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NOTE ON THE EFFECT OF TEMPERATURE UPON THE ACTION OF THROMBIN AND ANTITHROMBIN

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The main object of this note is to call attention to the marked augmenting effect of temperatures at or above the body-temperature upon the activity of antithrombin, and also to emphasize one important condition influencing the effect of high temperatures upon thrombin.

The effect of variations in temperature upon the time of coagulation has been studied by a number of observers, both as regards the clotting of normal blood and the coagulation of artificial plasmas or fibrinogen-solutions. In the case of normal blood the conditions are quite complex since several distinct processes come into play and these processes may be affected differently by the changes of temperature, the actual time of clotting being the resultant of their interaction. We have to consider, for instance, such changes as the disintegration of the platelets, the activation of the prothrombin, the neutralisation of the antithrombin and the final reaction between the thrombin and the fibrinogen. In consequence, perhaps, of this complexity of processes, and also because of the different methods employed, the results reported by different observers for normal blood have not been entirely concordant. All observers agree that between

0°C. and 20°C. rise of temperature is accompanied by a great acceleration of the time of clotting, the temperature coefficient for a range of 10°C. being quite large, 3 to 4. Between 20° and 30°C. some observers[1] find but little difference in the time of coagulation, while others,[2] making use of more delicate methods, state that there is a marked acceleration for a rise of temperature between these points, the temperature coefficient being approximately 2.5.

Between 30° and 40°C. there is a further difference in the reported results. Brodie and Russell got no variation in time of coagulation between these temperatures, Burker and also Addis found an acceleration, the temperature coefficient being from 1.4 to 1.7. Addis whose experiments were the most extensive states that beyond 42.5°C. coagulation is delayed, the temperature coefficient becoming negative. Observations upon artificial plasmas and upon fibrinogen-solutions have not given more uniform results, although the conditions involved would seem to be less complex than in shed blood. Rettger[3] states that with fibrinogen-solutions and thrombin (prepared by the method of Schmidt) the time of coagulation remains constant for variations of temperature between 17°C. and 41°C. Landsberg,[4] on the contrary, making use of similar preparations, reports that the time of coagulation is accelerated by rise of temperature up to an optimum which lies between 37° and 40°C. Beyond 40°C. the coagulation-time is somewhat delayed. My own observations tend to confirm the results reported by Landsberg. I made use of fibrinogen-solutions prepared in the usual way from oxalated plasma and thrombin solutions obtained by a method previously reported.[5]

It may be noted in this connection that the coagulation of fibrinogen-solutions by thrombin shows sometimes irregularities

[1] Lee and White: American Journal of the Medical Sciences, 1913, cxlv, 495.

[2] Brodie and Russell: Journal of Physiology, 1871, xxi, 403. Burker: Pflüger's Archiv, 1904, cii, 65. Addis: Quarterly Journal of Experimental Physiology, 1908, i, 305.

[3] Rettger: American Journal of Physiology, 1909, xxiv, 406.

[4] Landsberg: Biochemische Zeitschrift, 1913, l, 245.

[5] Howell: American Journal of Physiology, 1913, xxxii, 264.

which it is difficult or impossible to control, especially if minimal strengths of thrombin are used. If, for example, several mixtures of the same concentrations are made and are kept in a bath at a given temperature, it may happen that one of the specimens will vary markedly in its coagulation from the time shown by the majority of the preparations. Variations of this kind are due probably to some slight undetected difference in conditions whose influence is likely to be more evident the smaller the proportion of thrombin that is used.

In general, however, it was found that the optimum temperature of coagulation lies at 35°C. or between 30° and 35°, while at 40°C. there is a tendency toward a retardation or negative coefficient, which is never very marked and may be lacking in some cases, an important condition in this respect being again the proportional amounts of thrombin and fibrinogen used in the reaction. More decisive and interesting results were obtained in experiments upon the action of thrombin upon solutions of dried calcium-free blood plasma. The mode of making and using this dried plasma has been previously described[6] and need not be repeated here. With these solutions the time of coagulation is accelerated greatly between 0°C. and 20°C., the temperature coefficient being 3 or 3+, but between 20° and 35°C. the time of coagulation is not changed perceptibly. At 40°C. there is a very marked retardation—in fact, a permanent suspension of coagulation when the amount of thrombin used is not too large. This effect may be illustrated by the following experiment in which 1 drop of the thrombin solution was used to coagulate 10 drops of the plasma.

Temperature 20°C.	Coagulation-time between 5 and 10 minutes.
Temperature 25°C.	Coagulation-time between 5 and 10 minutes.
Temperature 30°C.	Coagulation-time between 5 and 10 minutes.
Temperature 35°C.	Coagulation-time between 5 and 10 minutes.
Temperature 40°C.	No coagulation within 3 hours, during which time the temperature of the bath was retained at 40°C.

[6] Howell: American Journal of Physiology, 1911, xxix, 187 and 1912, xxxi, i; also Archives of Internal Medicine, 1914, xiii, 76.

A specimen of the last solution removed after 2 hours to a temperature of 20°C. began to clot in 30 minutes and later formed a solid clot. Landsberg has observed a similar effect at body temperature in experiments made with a thrombin solution (Schmidt's method) and a magnesium sulphate plasma. His explanation is that the thrombin is adsorbed by some protein or proteins found in the plasma. In former papers I have given evidence for the existence of an antithrombin in blood-plasma, and the thought occurred that the striking difference in reaction at 40°C. between a thrombin-fibrinogen mixture on the one hand and a thrombin-plasma mixture on the other is due probably to the antithrombin present in the plasma. Direct experiments made to test this suggestion demonstrated that it is correct. In these experiments the action of the antithrombin was determined by its effect on selected mixtures of thrombin and fibrinogen exhibiting known coagulation times. For example, oxalated human blood plasma, freshly prepared, was heated to 60°C. and filtered. The antithrombin action of the filtrate was tested upon mixtures of thrombin and fibrinogen (dried plasma) at 20° and at 40°C. according to the following schema.

20°C. Thrombin solution drops	*Heated human plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time minutes*
4	1	15	10	4 (solid)
3	1	15	10	5 (solid)
2	1	15	10	10 (partial)

Without the addition of the drop of heated plasma similar mixtures all clotted firmly in 4 minutes.

40°C. Thrombin solution drops	*Heated humam plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time*
4	1	15	10	10 minutes (solid)
3	1	15	10	10 minutes (partial)
2	1	15	10	No clot in 5 hours.

This result was confirmed by other similar tests. It may be concluded that in mixtures in which the thrombin is not greatly in excess of the antithrombin an increase in temperature from 20°C. to 40°C. augments the action of the antithrombin to such an

extent that coagulation is greatly delayed or entirely prevented. In the plasma of circulating blood or lymph the prothrombin and antithrombin are present in such proportions that at room temperatures the coagulation of the cell-free plasma occurs very slowly, if at all. From the results here described it is evident that in such plasmas, at the body temperature, the action of the antithrombin must be favored to such an extent that the balance will be thrown safely to its side. Under the conditions that exist in the body in which the platelets and leucocytes remain intact and in which therefore there is no sudden increase in the content of the plasma in prothrombin and thromboplastin we can understand that the permanent fluidity of the plasma will be ensured by the protective action of the antithrombin.

THE EFFECT OF HIGH TEMPERATURES UPON THROMBIN

As pointed out by Rettger[7] solutions of thrombin made by Schmidt's method may be boiled for several minutes without losing wholly their power to cause clotting in fibrinogen-solutions. On the other hand it is very well known that if serum containing thrombin is heated to 60° the thrombic power is destroyed and when oxalated plasma is brought to the same temperature the prothrombin is destroyed or so changed that it can no longer be activated to thrombin. In the thrombin that I have prepared by acetone precipitation from saline solutions of washed fibrin I have had occasion to test at various times the degree of its thermolability, with the result that in some cases it has been destroyed apparently at relatively low temporatures, while in other cases even boiling has not removed entirely its characteristic action. Examination of these results has shown that the effect of high temperatures on the thrombin is greatly influenced by the character and amount of the salts present in solution.

Sodium chloride has a marked influence in this respect as is shown by the following experiments. A specimen of dry thrombin was dissolved in distilled water to make a 0.1 per cent solution. This solution was divided into three parts: A, con-

[7] Rettger: American Journal of Physiology, 1909, xxiv, 406.

taining no salt; B, containing sodium chloride to a strength of 0.5 to 1 per cent, and C, containing sodium chloride to a strength of 1 to 1.5 per cent. The three specimens were placed in a water-bath and heated to 60°C. for two minutes. Their action was then tested upon a freshly-prepared solution of fibrinogen in comparison with the same solutions unheated.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, heated, 2 drops gave clot in 20 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, heated, 2 drops gave clot in 60 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, heated, 2 drops gave clot in 95 minutes.

The same solutions were than heated to boiling over the flame for a minute and again tested upon the solution of fibrinogen.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated 4 drops gave clot in 3 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, boiled, 4 drops gave clot in 25 to 30 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, boiled, 4 drops gave no clot in 24 hours.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, boiled, 4 drops gave no clot in 24 hours.

It appears from these and similar experiments that an aqueous solution of pure thrombin free from salts is weakened but not destroyed by boiling, but that the presence of sodium chloride to a concentration of 1 per cent, more or less, renders the thrombin much more sensitive to high temperatures and effects its complete destruction at a temperature of 100°C.

No visible coagulation of the solutions was produced by the heating and the way in which the salt exerts its influence upon

the thrombin remains undetermined. It may be added that boiling aqueous solutions of thrombin, free from salts, for 5 minutes, is not sufficient to destroy their action completely. They still cause clotting of solutions of fibrinogen although the time is delayed. If the solutions are kept in a bath of boiling water for 30 minutes some remnant of activity is still maintained, for when added to solutions of fibrinogen they cause, after some time, a delicate membraneous clot.

SUMMARY

1. At temperatures approximating the body-temperature the action of antithrombin is greatly augmented. It is probable that this action is of importance in ensuring the fluidity of the circulating blood in animals like the mammals in which the content of antithrombin in the blood is small.

2. The effect of high temperatures (60°–100°C.) in weakening or destroying the action of thrombin is accelerated greatly by the presence of small amounts of neutral salts (NaCl).

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.
NO. 255.

THE ORIENTATION OF A HOLOTHURIAN BY LIGHT

W. J. CROZIER[1]

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I. In a paper dealing with the sensory reactions of Holothuria surinamensis, which is to appear in the Zoologischer Jahrbücher, I have shown (Crozier, 1914 [?]) that the analysis of echinoderm reactions to light given by Mast (1911, pp. 211–213) fails when applied to Aspidochirote holothurians, since (1) these animals move only with the anterior end in advance, and (2) they *orient away* from the light, yet react *negatively* to shading. It may not be out of place to publish a more detailed account of the photic orientation of another holothurian, H. captiva, which exhibits this reaction in an even more clearly defined manner.

This study was carried out at the Bermuda Biological Station for Research during the summer of 1914. For the use of the facilities of the station, my best thanks are due the Director, Prof. E. L. Mark.

II. Holothuria captiva Ludwig is a small dark-green Aspidochirote holothurian,[2] which is relatively common in certain localities in the Bermudas. It is found exclusively, during the daytime at least, clinging to the under sides of slabs of stone on rather exposed shores, just under low water mark. Individuals ranging in length from 4 to 100 mm. are obtainable during the months of June and July.[3] Experiments were made upon animals covering this range in size.

[1] Contributions from the Bermuda Biological Station for Research. No. 34.

[2] The paper on H. surinamensis contains general observations on the behavior of H. captiva.

[3] The majority of the younger specimens were found at the entrance to Hungry Bay.

The bilateral symmetry of this form is more strongly pronounced than is the case with the related H. surinamensis and H. rathbuni, though, as might be expected from the near structural affinity of all three, they show many points in common. The accentuation of physiological polarization in H. captiva is due mainly to the fact that the ventral trivium (which alone bears tube feet) is, even in very young animals, more flattened and sharply marked off from the lateral body surfaces than in the other two species; a minor factor is the greater obliquity of the oral plane, which runs obliquely ventrad and posteriad. This bilaterality is further brought out by the relatively greater rigidity of the body; lengthwise spiral twisting, common in H. surinamensis, does not occur here, and the main tendency of the body, in the absence of special stimuli, is to preserve the straightness of its horizontal axis.

The anus is situated dorsally, and when the animal is intensely stimulated, the brim about it may be elevated into a chimney-like tube, capable of being directed from side to side. This is connected with the great development of Cuvierian organs in this species, for the tube, being directed toward an irritated point on the body, controls in a general way the direction in which these organs are discharged.

In the youngest stages found (4 to 10 mm. long) the pigmentation of the body differs from that of the larger specimens, since in the very small animals the whole body is of a light green color, while in the older ones this hue persists only on the trivium, in the tentacles, and on the tips of the dorsal papillae, the dorsal and lateral surfaces becoming deep olive green. Even in the youngest specimens the tentacles are less highly colored than is the rest of the body. Darker pigmentation first becomes evident about the bases of the papillae, in animals about 8–9 mm. long; it is due, largely, to an increase in the amount of the lighter pigment, though another substance, dark brown and chemically distinct from the former, is also concerned. Some chemical characteristics of the green pigment and its possible rôle in photoreception have been treated of in the paper above referred to (Crozier, 1914 [?]), and will be further considered below.

III. Holothuria captiva ordinarily lives in dark situations. To keep it in a healthy condition for any length of time in the laboratory, it must be shaded. Prolonged exposure to even moderately bright diffused light exerts a distinctly toxic effect. It is photokinetic. The whole surface of the animal is sensitive to light. Tests made with the aid of an apparatus designed to produce small areas of light[4] gave results entirely consistent with those previously found for H. surinamensis. The tentacles reacted negatively, as shown by their partial contraction and by more or less undirected waving movements. The pedicels (when the spot-light was intense) became detached and waved about. The papillae were caused to collapse. Every point on the body-surface reacted negatively to sufficiently strong light by local in-sinking.[5] The order of sensitivity of the parts of the animal is: anterior end > posterior end > pedicels, papillae > mid-body surface.

The method of orientation to horizontal light of not too great an intensity, coming from a single source, will be apparent from an examination of the sample trails given in the text figures and in the plate. The tests, with the exception of those shown, were made by admitting sunlight, or light from a 40 c.p. tungsten filament, through a diaphram into a blackened box containing the holothurians in a flat-sided glass aquarium. Sketches of the moving animals were secured, in a dark-room, by having the aquaria mounted on short legs above a table, so that outlines of their successive positions during orientation could be conveniently traced on paper placed beneath them. The photographs were made with the holothurians exposed to two sources

[4] This apparatus, modified from one devised by Dr. B. M. Patten, consists essentially of a small pocket electric flash-light with a tungsten filament mounted, in place of the ocular, on a microscope tube provided with a low power objective (A* or "3"). Diaphrams are readily adjusted between the electric bulb and the objective, and if necessary within the lens-system, in such a way as to give beams of light of any desired cross-section and devoid of halos. The instrument as thus constructed is self contained and convenient to handle.

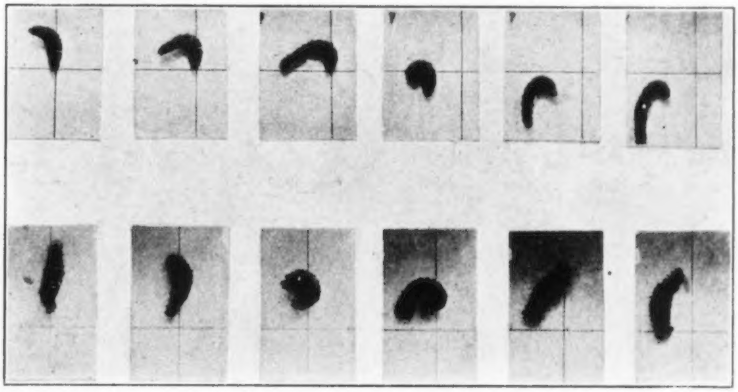
[5] There is no question of a heat effect being involved, since I have found that Holothuria is not equipped with anything which might properly be termed a temperature sense.

of light, diffuse daylight and more intense sunlight reflected horizontally by a mirror. The movements of orientation are sufficiently slow to permit the use of an ordinary "kodak," clamped to an upright for obtaining records. No differences in the mechanism of orientation were noticeable under these slightly different experimental conditions.

If H. captiva is suddenly subjected to horizontal light parallel to its long axis thrown on its anterior end, the pedicels of that region are released and the anterior half of the body is swung

Series A. PLATE I

3:27 3:27.7 3:28 3:29 3:29.3 3:29.7



9:42 9:43 9:43.5 9:44.5 9:45.7 9:47.5

Series B.

EXPLANATION OF PLATE

Stages in the orientation of Holothuria captiva by sunlight parallel to its long axis incident upon its anterior end. In Series A moderately bright light was used, in Series B, light of lower intensity. The crossed lines shown were on a card placed beneath the aquarium. The rate of orientation can be judged by the relation of the animal to these coördinates at the times indicated opposite each picture.

sharply to one side (Plate I; and figs. 1, 2, 3). The swinging movement continues until the anterior end is turned as far away from the light as possible; the anterior end is then extended somewhat and re-attached; the posterior end is then frequently drawn forward by contraction of the anterior body muscles (Plate I,

figs. 2, 3); the turning away from the light continues until the animal is finally crawling in the direction of the beam. Its rate of locomotion then decreases, for the area exposed to the light is lessened; moreover, the sensitivity of the posterior end is less than that of the anterior one. Similar movements follow the application of light to the side of the animal (fig. 4). In every case the first movement is away from the light. It is only occasionally that anything which might, by any stretch of the imagination, be termed a trial movement appears. One such case is here recorded (fig. 2). It is readily accounted for by the tendency to persistence which echinoderm movements

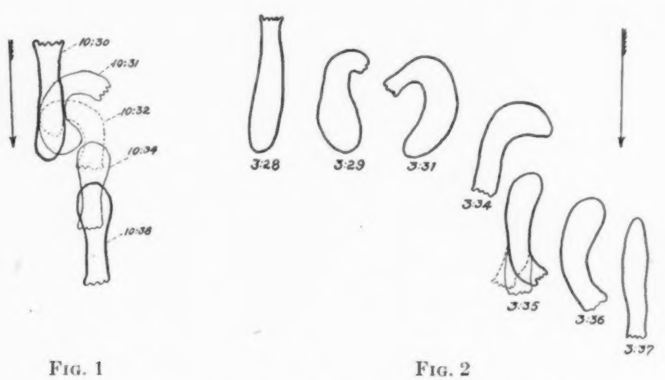


Fig. 1 Fig. 2

Fig. 1 Stages in the orientation of H. captiva to moderately bright sunlight. Arrow shows direction of the light. The time at which each recorded observation was made is given.

Fig. 2 Conditions as in figure 1; a case which shows pseudo trial movements. The successive outlines have been shifted laterally for the sake of clearness.

in general display. The first turning movement was carried too far (3:35), and stimulation on the other side forced the anterior end to swing back; it is to be noted that these pseudo "trial movements" are not maintained.

The rapidity of orientation varies with the light intensity employed, a higher intensity giving a more rapid orienting effect (see Table 1).

Direct sunlight, or electric light of more than 200 c.m. intensity, has an almost immediate toxic action (Table 2). Animals exposed to light of these intensities were within 15 to 30 minutes caused to eviscerate through the anus or through dehiscent openings in the body wall.[6] Previous to the production of this moribund condition the animals attempted to orient, but before this reaction was completed they moved aimlessly, and later withdrew the tentacles, pedicels and papillae. After a quarter of an hour in this state very few recovered when removed to more favorable conditions.

TABLE 1

Time required for the completion of orientation by horizontal light parallel to the long axis of the animal acting on the anterior end, for individuals of different sizes; (a) with diffuse daylight, (b) with moderately bright direct sunlight. Tests were made at least an hour apart.

ANIMAL		ORIENTATION TIME, MIN.	
No.	Length, mm.	a	b
1	6	51	30
2	40	6	3
3	60	7	3
4	70	10	4
5	85	11	6

TABLE 2

Number of successive reactions to shadows, cast at 0.5 minute intervals, obtained from the anterior end of Holothuria captiva of different sizes before exhaustion; (a) in diffuse sunlight (b) after being in bright sunlight for 10 minutes.

ANIMAL		NO. OF REACTIONS	
No.	Length, mm.	a	b
1	25	3	0
2	40	7	0
3	43	3	0
4	44	4	0
5	45	4	0
6	53	18	2
7	56	8	1
8	58	16	1
9	62	9	1
10	68	19	4
11	75	19	3

[6] Under these conditions evisceration through the anus took place without the discharge of the Cuvierian organs. Taken in connection with the results of certain of my experiments, this leads me to doubt the entire correctness of Mines's explanation of the discharge of these organs as due to internal pressure. A variation of the experiment he performed on H. nigra (Mines, 1912) consisted in artificially raising the internal pressure of H. captiva by pressing on it. Cuvierian organs may thus be caused to come out through the anus at will, but their appearing and the mode and extent of their subsequent elongation are then decidedly not normal.

IV. The only conclusion permissible from the above experiments is that the behavior of Holothuria captiva toward horizontal light is a clear and definite example of negative phototropism, in the sense intended by Loeb. The animal is sensitive to light; it is one of the few echinoderms thus far investigated which possesses a strong physiological polarity as regards movement and a functionally bilateral structure; it is compelled so to adjust itself in a field of light that the effect on its opposite sides is equalized.

What is the significance of these findings in interpreting the photic reactions of starfishes, ophiuroids and sea urchins which,

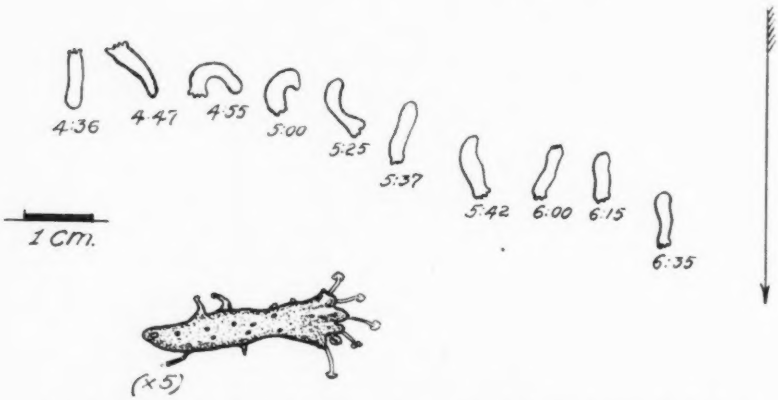


Fig. 3 Details in the orientation of an animal 6 mm. long, of which an enlarged dorsal view is given. Outlines separated laterally to avoid confusion of lines.

in contrast to H. captiva, move toward, or away from, the light without orientation? In the forms which have hitherto been studied, there is no pronounced structural or physiological prominence of an anterior end (cf. Cole 1913). They may, then, be regarded as animals which are phototropic, but in which the part which is the effective anterior changes with the altering of external (and internal) conditions. This explanation also

applies to holothurians like Thyone (Pearse 1908)[7] and (in my experiments) Cucumaria punctata. These animals are not nearly so sensitive as H. captiva, nor is their structural bilaterality so marked; in particular, functional pedicels are in Thyone found all over the body, and in Cucumaria on all five radia.

V. Holothuria captiva is likewise sensitive to shading (Table 2). It reacts negatively over its whole surface to sudden decrease in light intensity, but is not reactive to an increase of intensity. The tentacles are the most sensitive; the order of sensitivity of the parts of the animal is the same as that for the direct action of light. It is probable, therefore, that a single

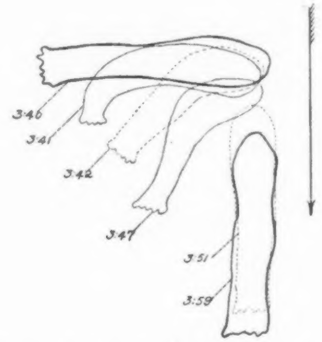


Fig. 4 Orientation by lateral light.

photoreceptive system is present, which is capable of responding to both kinds of stimulating agencies.

A detailed review of the literature on "differential sensitivity"[8] would consist almost entirely in a mere catalog of the reactions of a variety of animals to shading, or to increase in light intensity, or, rarely, to both. Such reviews may be found in the books of Mast (1914), Kafka (1914), and similar works. With the excep-

[7] Scott (1914) speaks of Thyone as being "oriented by light," but seems to use the word in a very loose sense.

[8] "Differential sensitivity" is personally preferred to Bancroft's "differential sensibility" (Bancroft, 1913); both are equivalents of "Unterschiedsempfindlichkeit."

tion of v. Uexküll's papers (1897, 1900), there are very few recorded facts of physiological significance, so far as an understanding of the mechanism involved in stimulation is concerned. It may be pointed out, however, that writers on this subject—Rawitz (1888), Nagel (1896), and particularly Hargitt (1909) and Verworn (1913)—have been troubled by the fact that a shadow, "the negation of light," can produce a positive sensory effect. The difficulty, as is clear from the discussion of Hargitt (1909), and Verworn (1913, pp. 41 et seq.), results from a too superficial view of the nature of a "stimulus." This may be seen in the following quotation from Verworn (op. cit., p. 44):

> . . . . It is altogether impracticable to define the stimulus itself in relation to the nature of the effect which the stimulus has upon the substances in the system. One can only appreciate the nature of stimulation in relation to the vital conditions and without considering the nature of the action of the stimuli on the living substance.

So far as I can discover, this statement is physiologically meaningless. It is not out of place, in this connection, to insist upon the simplicity of the view that when a sensory cell is aroused to activity the real *stimulus* is the changed physico-chemical condition within the cell, or at its surface membrane.

The integument of Holothuria is provided with pigment cells, in relation with which there terminate fibers of the radial nerve strands. Polara (1906) suggested that these were the organs of a diffuse photic sense. If we picture, possibly located in these cells, a balanced system including (1) a photosensitive material, (2) the precursors which in the normal course of metabolism go to produce it, and (3) the products of its photolytic decomposition, which may or may not be identical with its precursors, we have all the essentials of a mechanism with which to account for the photic reactions of Holothuria. The photolysis of the substance provides the stimulus for photokinetic reactions and phototropism, while the abrupt cessation of this photolytic action, or the re-institution of reconstructive reactions previously inhibited by light, is the stimulus for the shading reflex. This

view is consistent with the electrical behavior of the excised vertebrate eye under similar conditions (Einthoven and Jolly, 1908).

I was at first of the opinion that possibly the green skin pigment was itself the photosensitive material. It is fluorescent, with a bluish-green light like uranium glass, and water or alcohol extracts containing it are bleached by exposure to sunlight and air. But this conception must be modified in view of subsequent experiments. Fresh neutral or alkaline watery extracts of the skin of Holothuria contain this pigment and an abundant supply of a catalase-like enzyme. They are not bleached rapidly enough, even with the addition of hydrogen peroxide, to warrant the belief that the pigment is normally decomposed by light to any great extent. But such preparations show a notable acceleration in the evolution of oxygen from hydrogen peroxide when they are exposed to bright sunlight, which disappears on return to the shade. It is not too extreme to suggest that there is some relation between this increase in peroxide catalysis and the fact that nerve processes terminate in connection with the surface of the pigment cells. R. S. Lillie (1913) has shown that in frog leucocytes the most rapid oxidations occur at the nuclear and plasma membranes, and that these oxidations are increased by stimulation.

The fluorescent pigment therefore probably acts merely as a sensitizer. The theory of the intimate connection of this pigment with photoreception is supported, not only by the widespread occurrence of fluorescent substances in animal photosensitive organs, and the well-known influence of such materials in increasing the toxic action of light when they are injected, but also by the following observations which I have made upon cases occurring in nature:

(*a*) H. surinamensis is a nocturnal animal. In its normal habitat it comes during the night to the surface of the sandy mud in which it lives. When found, as is occasionally the case, among rocks, the surface of the body, excepting the tentacles and podia, is covered by a thin firm layer of dark silt held by a mucoid secretion. In the summer of 1914 I secured four individuals of this species which were found on the upper surface

of stones in fairly bright sunlight. They were 6 to 10 cm. in length. These specimens were devoid of greenish coloring matter, the only pigment visible being the dark brown melanoid which usually accompanies the fluorescent material. Though kept in the laboratory for some days, these animals were found to be totally insensitive to shading and only vaguely photokinetic. They did not orient to light.

(*b*) H. surinamensis and small H. captiva are sometimes found having unmistakably regenerated anterior or posterior ends.[9] Such portions are notably deficient in pigmentation and at the same time give evidence of a lower sensitivity to light and shading than that possessed by the normally pigmented tissues.

(*c*) In the three Bermudan species of Holothuria the order of increasing sensitivity to light and shading—H. rathbuni < H. surinamensis < H. captiva—is exactly that of the relative increasing amount of fluorescent green pigment in their skins.

(*d*) It is a general rule, certainly true of holothurians in other responses, that the activity of an animal is inversely proportional to its size. Comparing the number of shading reactions obtained from animals of different sizes (Table 2), it will be seen that the smaller individuals, which react, in general, more vigorously, are more quickly exhausted. The pigmentation of the very young H. captiva (about 4–6 mm. long), however, is less dense than that of older specimens, and these are much less sensitive to photic stimulation, both in the shading reflex and in photokinesis. The relative times occupied in orientation to light of standard intensity will illustrate this point (see the trails in figures 1 and 3, and Table 1).

[9] These structures in H. surinamensis can be duplicated by regeneration after experimental cutting. In nature they are probably the result of autotomous bisection. I can confirm for H. captiva the statement of Dalyell (1851) that young holothurians undergo spontaneous self division. Larger specimens of the species last mentioned do not show naturally occurring regeneration and do not regenerate in the laboratory. In this respect H. captiva resembles the majority of the genus (Torelle, 1909).

SUMMARY

1. Holothuria captiva Ludwig is photokinetic. Its whole surface is sensitive to light and to shading. It gives no reaction to an increase in light intensity. The order of sensitivity of its parts is: anterior end > posterior end > podia > mid-body surface.

2. Light above 200 c.m. intensity exerts a distinctly toxic influence.

3. Like H. surinamensis, the animal moves only with the anterior end in advance.

4. Unlike starfishes, sea-urchins, and less pronouncedly bilateral holothurians, it is oriented by light. It is negatively phototropic.

5. The photoreceptive mechanism includes the action of the green fluorescent integumentary pigment as sensitiser.

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THE EFFECT OF RADIANT ENERGY ON THE LENS AND THE HUMORS OF THE EYE

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The object of this investigation was to determine what effect is produced on the lens and on the aqueous and vitreous humors by radiation from a quartz mercury vapor lamp, a Mazda nitrogen lamp, an electric furnace and the sun. The lenses and the aqueous and vitreous humors used were from the eyes of the pig and ox.

I

QUARTZ MERCURY VAPOR LAMP

A Cooper-Hewitt quartz mercury vapor lamp operating at 170 volts, 3.3 amperes and 2400 candle power was employed in the following experiments. Lenses were introduced into quartz tubes 1 cm. in diameter, 10 cm. in length with walls approximately 0.5 mm. in thickness. The diameter of a lens was slightly greater than the diameter of a tube so that the tubes were completely filled in an horizontal direction. A quartz tube containing lenses was filled with egg white. Similarly other tubes were filled with vitreous humor, aqueous humor, blood serum and distilled water. The tubes containing the different kinds of media were closed with rubber stoppers and placed horizontally 1 cm. beneath the surface of running water in a tank under the burner. The burner operated at 5 cm. from the surface of the water.

[1] I wish to express my thanks to Dr. E. P. Hyde, Director of Nela Research Laboratory, for the privilege of working in his laboratory and to the members of the staff for their help in this investigation.

The egg white in the quartz tube began to coagulate after 15 minutes' exposure to the light. At the end of 72 hours' exposure the egg white was a firm coagulum but the immersed lens was as transparent as at the beginning of the experiment. At the end of 120 hours' exposure a slight opacity had been produced in the part of the cortex of the lens directly exposed to the light. Parallel experiments were carried out using glass tubes but on account of the putrefaction of the material the use of the glass tubes was discontinued. Putrefaction, however, did not take place in the glass tubes for 40 or 50 hours in which time there was scarcely any precipitation of the egg white. This is in keeping with the well known fact that glass transmits very poorly the short wave lengths of the spectrum to which the coagulating property of the quartz mercury vapor lamp is due.

The vitreous humor in the quartz tube became slightly turbid after 24 hours' exposure to the light while the transparency of the lens immersed in it was unaltered. At the end of 72 hours' exposure the vitreous humor had become opaque while the transparency of the lens was still unchanged except for the part directly exposed to the light. This part was covered by a thin cloudy film. A quartz tube filled with clear vitreous humor but containing no lens was exposed to the light for 150 hours. At the end of this time there was a very slight cloudiness in the material in marked contrast to the opacity of the humor in the preceding experiment in which the lens had been immersed during the 72 hours' exposure to the light.

The aqueous humor in the quartz tube in which a lens was immersed became slightly turbid after 24 hours and opaque after 72 hours but the transparency of the immersed lens was very little affected. A quartz tube filled with aqueous humor but containing no lenses was exposed to the light for a period of 150 hours with absolutely no change in the clearness of the liquid. As in the case of the vitreous humor this clearness was in marked contrast to the opaqueness of the aqueous humor in which a lens had been immersed during the experiment.

On exposure of the quartz tube containing the lenses immersed in distilled water the liquid became turbid in 45 minutes.

After 72 hours' exposure the transparency of the lenses was scarcely affected.

Lenses immersed in blood serum contained in quartz tubes were exposed to the light. The serum was obtained by centrifugalizing defibrinated pigs' blood. After 15 hours the serum became slightly turbid and after 72 hours it was opaque. At that time there was a slight cortical opacity on the part of the lens directly exposed to the light.

II

MAZDA NITROGEN-FILLED LAMP

A 750 Watt, nitrogen-filled Mazda lamp operating at 0.6 Watt per candle power was used. By means of a plano-convex glass lens having a diameter of 4 inches and a focal distance of approximately 12 inches an image of the filament of this lamp was focused about 1 mm. below the upper surface of the materials used. The materials were similar to those on which the radiation from the quartz mercury vapor lamp was studied, viz., lenses immersed in egg white, in vitreous humor, in aqueous humor, in blood serum and in distilled water. The lenses immersed in these media were exposed to the light as described both in quartz tubes and directly in open mouthed vessels. In no case was any apparent effect produced either in the media or in the immersed lenses.

III

ELECTRIC FURNACE

The electric furnace employed was $1\frac{3}{4}$ inches in diameter, $2\frac{1}{2}$ inches in depth with a heating coil of platinum wire imbedded in clay and operating at a temperature of approximately 1000°C. The materials were similar to those used in the experiments with the quartz mercury vapor lamp, viz., lenses immersed in egg white, in vitreous humor, in aqueous humor, in blood serum and in distilled water. A glass tube 5 cm. in length and $1\frac{1}{2}$ cm. in diameter was filled to a depth of 3 cm. with each of these media

in turn. A float, made of thin circular cork, cut to fit the tube, with a hole in the center approximately 1 cm. in diameter was prepared. The lens was fitted into the hole in the float and held in position by means of a small piece of gauze stretched across the under side of the float and attached to its edges. The float with the attached lens was introduced into the test tube onto the surface of the medium for the experiment in question. With such an arrangement the float and the entire lens except its very upper sûrface were covered with the medium. The tube thus prepared was clamped into position in a tank of running water so that the material within the tube was 1 cm. below the surface of the running water outside the tube. The electric furnace heated to an intense red heat, and suspended over the mouth of the furnace, was 15 cm. from the top surface of the lens. The lens was exposed to the radiation from the furnace at this distance for 24 hours. There were indications at the end of this time of drying at the surface of the lens, but in none of the experiments was opacity produced when the furnace was 15 cm. from the material. When the furnace was operated at a distance of 5 cm., the upper surface of the lens was made opaque in 15 to 20 minutes. On placing a thermometer on the surface of the lens under these conditions there was found a rise of temperature to as much as 65°C. The obvious conclusion is that the opacity was due to the heat effect and not to the radiation.

IV

THE SUN

Apparatus similar to that used in the experiments with the electric furnace was employed. The tank of running water was replaced by a vessel containing a mixture of ice and salt. The image of the sun was focused on the lens by means of a plano-convex glass lens 4 inches in diameter and having a focal distance of about 12 inches. In this way opacity of the lenses could be produced in a few minutes, but in every case the thermometer showed that there was a great rise of temperature in

and around the lens. Presumably the opacity in this case is due to the heat effect as in the case with the electric furnace.

These experiments show that it is practically impossible to precipitate the native protein of the lens and of the aqueous and vitreous humors of the eye by means of radiant energy. In confirmation of the work of others they also show that egg white and blood serum are very easily coagulated by ultra violet radiation, while the longer wave lengths in the visible spectrum and in the infra red have no such effect. They also show that the protein of the lens, extracted by means of distilled water and by means of the aqueous and vitreous humors, is easily precipitated by the short wave lengths of the spectrum.

Dreyer and Hanssen[2] and others have shown that practically all the ordinary proteins can be precipitated with more or less ease by ultra violet radiation. In view of the experiments just described it would seem that the protein of the lens offers a conspicuous exception to the generalization in that it is practically impossible to coagulate its protein by means of ultra violet rays. In the development of cataract the lens becomes opaque and the most plausible assumption is that this opacity is due to the precipitation of its protein. As a matter of fact it is difficult to understand how opacity could be produced in any other way.

Analyses[3] of normal and cataractous human lenses show that in cataract there is a great increase over the normal in the amount of certain salts. This fact suggested that it might be possible to alter the lens protein by means of these salts so that it would be possible for radiant energy, particularly the ultra violet, to coagulate the altered protein. The result of the analyses of many hundreds of normal and cataractous lenses may be seen in the accompanying table which gives the average result as estimated for a single lens. The human cataractous lenses were obtained from different parts of the United States and from India.

[2] Dreyer and Hanssen: Comptes Rendus, 1907, cxlv, 234.
[3] Burge: Archives of Ophthalmology, 1909, xxxviii, 447.

TABLE I

	AVERAGE DRY WT. OF ONE LENS	AVERAGE WT. OF ASH OF ONE LENS	PERCENT WT. OF ASH TO DRY WT.	PERCENT OF K IN ASH	PERCENT OF Ca. IN ASH	PERCENT OF Mg. IN ASH	PERCENT OF Na. IN ASH	PERCENT OF Si. IN ASH
	mgr.	*mgr.*						
Normal adult human lens	58.99	1.40	2.30	38.80	?	?	?	0
Normal adult pig's lens	137.70	3.40	2.45	34.30	0.08	1.20	6.67	0
Embryo human lens	15.47	0.25	1.60	30.80	?	?	?	0
Cataract human lens (United States)	34.42	0.58	1.68	9.80	12.50	8.00	23.82	0
Cataract human lens (India)	92.30	1.52	1.64	5.81	6.00	1.6	25.06	3.63

This table gives the percentage composition as estimated for a single lens. It may be seen from the table that in the human cataractous lens the percentage of potassium in the ash is greatly reduced while the percentages of calcium, magnesium and sodium are greatly increased over the amounts existing in the normal lens. In cataractous lenses obtained from the United States there was no indication of silicate while those from India contained distinct amounts of the silicates of potassium, calcium and possibly of sodium. What conditions caused such a marked deposition of silicate in the cataractous Indian lenses cannot be stated.

With the results of these analyses in mind and the facts that cataract is found as a complication of diabetes and that its occurrence is frequent among glass blowers experiments were performed using the same apparatus as in the preceding experiments but with $\frac{M}{100}$ calcium chloride, $\frac{M}{100}$ dextrose, $\frac{M}{100}$ potassium chloride and very dilute solutions of magnesium chloride and of sodium silicate as media for the lenses in place of the egg white, the serum, the eye humors and distilled water employed in the previous experiments. It was found that these strengths of solutions had no effect upon the transparency of lenses immersed in them for 72 hours, or longer, in the dark or in ordinary day light. The exposure of lenses immersed in similar solutions to the radiations from the nitrogen-filled Mazda lamp, from the

electric furnace and from the sun produced no change in the transparency of the lenses, as also had been the case when egg white, serum and the eye humors were used as media. However when lenses immersed in the sugar and salt solutions were exposed to the radiation from the quartz mercury vapor lamp quite different results were obtained.

The lenses were placed in quartz tubes containing the different media. The tubes were stoppered and permitted to stand for 2 hours. At the end of this period the tubes containing the lenses and media were placed horizontally under the burner in a tank of running water 1 cm. beneath the surface of the water.

On exposure of the quartz tube containing the lenses immersed in $\frac{M}{100}$ calcium chloride the liquid became turbid in 15 minutes. The part of the lens directly exposed to the light became more and more opaque and at the end of 72 hours the half of the lens directly exposed to the light had become an opaque mass while the opposite half remained almost perfectly transparent.

Lenses that had been standing in $\frac{M}{100}$ dextrose for 2 hours were exposed to the light. The liquid in the quartz tube containing the lenses became turbid in 40 minutes. The part of the cortex of the lens on the side next to the light had become somewhat opaque in this time. This opacity after 72 hours' exposure had increased until it was about .5 mm. in depth, while the part of the lens away from the light was very slightly opaque.

On exposure of the tube containing lenses in $\frac{M}{100}$ potassium chloride the liquid became turbid after 50 minutes and after 72 hours there was a gross suspension in the liquid. The part of the cortex on the side next to the light had become at this time opaque while the opposite part of the lens remained transparent.

Lenses were placed in approximately $\frac{M}{100}$ magnesium chloride solution for 2 hours. On exposure to the light the liquid became turbid in about an hour. The part of the lens directly exposed to the light became a dense opaque mass after 72 hours' exposure

while the part not directly exposed to the light had become only slightly opaque.

Lenses were placed in a very dilute solution of sodium silicate for 2 hours. On exposure the same results were obtained as with the magnesium chloride.

These experiments show that it is possible to modify the lens protein in such a way that ultra violet radiation will precipitate it, whereas when the protein is not modified the radiation will not prĕcipitate it.

It is possible to produce opacity of lenses by immersion in any of the above solutions but to do this it is necessary that these solutions be much stronger than those used in the experiments given above and also much stronger than ever occurs in the living animal. For example, it requires a 15 per cent potassium chloride solution to produce nuclear opacity and a 10 per cent dextrose or 1 per cent calcium chloride solution to produce cortical opacity. In trial experiments I had found that lenses immersed in a 12 per cent potassium chloride solution never developed nuclear cataract, those immersed in a 13 per cent potassium chloride solution occasionally developed it after about 12 hours and those immersed in a 15 per cent potassium chloride solution always developed this opacity after about 6 hours. The transparency of the cortex of the lenses in which nuclear opacity had developed was not noticably effected. The fact that 15 per cent potassium chloride will produce nuclear opacity in a lens suggests the possibility of a relation between the production of this type of cataract and this salt. The following experiments were devised to determine if the short wave lengths would influence the production of nuclear cataract by potassium chloride.

Lenses were introduced into a 12 per cent potassium chloride solution and were exposed to the radiation of the quartz mercury vapor burner. No opacity of the nucleus was produced after 12 hours either in the lens exposed to the radiation or in the control experiment in which the lens was not exposed. Several lenses were introduced into a 13 per cent solution of potassium chloride and were exposed to the radiation. After 12 hours the nuclei of three of the lenses became opaque while the

nuclei of the remaining lenses were transparent. In the control experiment in which lenses were immersed in the same strength of potassium chloride but not exposed to the radiation the nuclei of two of the lenses became opaque, while the nuclei of the four remaining lenses were transparent. Lenses were introduced into a 15 per cent potassium chloride solution and were exposed to the radiation. Opacity of the nuclei of all of these lenses developed after about 6 hours. The nuclei of the lenses in the control experiment became opaque in about the same time.

From these experiments it may be concluded that ultra violet radiation cannot produce nuclear opacity in lenses immersed in potassium chloride of a strength slightly less than that of a solution capable of producing nuclear opacity itself, and that ultra violet cannot hasten the production of this opacity when the lenses are in solutions which are of themselves strong enough to produce it.

In the above experiments using potassium chloride the liquid surrounding the lens became turbid after a short period. It might be objected that the ultra violet radiation had no effect on the development of nuclear opacity because the radiation did not reach the nucleus, being absorbed or scattered by the opaque liquid surrounding the lens. Experiments were carried out with the view of meeting this objection. A lens was cut in equal parts, one-half of the nucleus being left in each half of the lens. One of the halves was wedged into a quartz tube so that the cut surface was pressed firmly against the side of the tube. The tube was then filled with a 12 per cent potassium chloride solution, stoppered and placed under the burner with the nucleus exposed directly to the radiation. The exposure was continued for 48 hours. At the end of this time the nucleus showed no indication of opacity. A similar experiment was carried out using 15 per cent potassium chloride. The opacity of the nucleus of the half of the lens exposed to the radiation developed after about 6 hours. The opacity of the nucleus in the control experiment developed in about the same time.

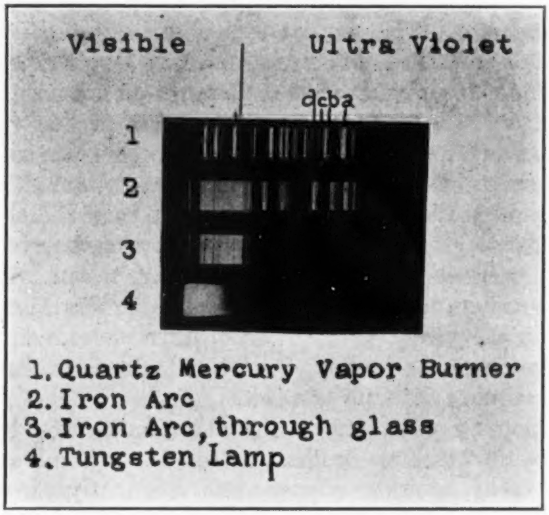
Another fact against the assumption that potassium chloride is concerned in the production of nuclear cataract is found in

the analyses of the nuclei of cataractous lenses. These nuclei did not show any increase in the content of potassium over the normal lens, but in keeping with cataractous lenses generally show a marked decrease in potassium. All this evidence would seem to indicate that potassium salts are not concerned in the production of nuclear cataract. On the other hand experiments carried out in the course of this investigation indicate that calcium, magnesium, dextrose and silicate may play an important rôle in the production of cataract in that these substances modify the lens protein in such a way that ultra violet radiation can precipitate it.

Experiments were carried out using the Fuess quartz spectograph in order to determine which wave lengths, emitted by the quartz mercury vapor lamp, caused the precipitation in the modified lens protein.

Clear fresh egg white was introduced into a quartz cell. The cell was made of two quartz disks 4 cm. in diameter and 1.2 mm. thick. These disks were separated by a ring of hard rubber 0.8 mm. thick with an inside diameter of 3.8 cm. By means of the spectrograph the spectrum from an 800 candle power quartz mercury vapor lamp was focused on the egg white in the cell. The slit of the spectrograph was 1 mm. wide and the burner was placed 3 cm. from the slit. The coagulation of the egg white began after 15 hours towards the extreme end of the ultra violet in the form of a well defined band of white coagulum corresponding in position to that of an intense band of the spectrum marked "a" on the accompanying photograph. Although "a" appears as one intense band it is in reality composed of three fused bands of wave lengths $265.2\mu\mu$, $265.3\mu\mu$ and $265.5\mu\mu$, respectively. At the end of 24 hours three more bands of coagulated egg white were to be seen corresponding in position to three other bands in the spectrum, "b," "c" and "d," wave lengths $289.3\mu\mu$, $296.3\mu\mu$ and $302.1\mu\mu$, respectively. From these results it would appear that the effective region in producing coagulation of the egg white is between $265\mu\mu$ and $302\mu\mu$ and that the most effective region is around $265\mu\mu$ for the quartz mercury arc.

Five lenses were extracted with 0.25 per cent calcium chloride for 2 hours. At the end of this time the clear liquid was introduced into the same quartz cell and exposed in the same manner as the egg white had been. At the end of 15 hours there could be seen one delicate white band of coagulated lens protein, fairly well defined, corresponding in position to the same intense band in the ultra violet region where the egg white was first coagulated, marked "a" in the photograph. At the end of 24 hours' exposure there had appeared two more delicate white bands, more or less



Photograph of Spectograph Plate*

well defined, of coagulated lens protein. These two bands corresponded in position to the same two bands in the ultra violet region of the spectrum where the egg white was coagulated after 24 hours, marked "b" and "c" on the photograph. In place of the other band where the egg white was precipitated, marked "d" on the photograph, there was an ill-defined hazy precipitation of lens protein. The same conclusion can be drawn regarding the precipitation of modified lens protein as was drawn regarding the precipitation of egg white, viz., that the effective

*By M. Luckiesh.

region is from $265\mu\mu$ to $302\mu\mu$ inclusive, and that the most effective region lies around $265\mu\mu$.[4]

It is known that cataract may be a complication of diabetes. In this disease it is also known that sugar is increased in the blood and in the body fluids and presumably in the eye media. This increase of sugar, however, is not of sufficient strength of itself to produce opacity of the lens so there must be another factor involved. The experiments cited in this paper showing the effect of ultra violet radiation on the protein of the lens modified by sugar would suggest that ultra violet radiation is the other factor. The normal lens absorbs wave lengths between $350\mu\mu$ and $300\mu\mu$ and transmits wave lengths longer than these. These absorbed short wave lengths do not normally produce opacity in the lens. The experiments show that very weak solutions of sugar can modify the protein of the lens so that the absorbed short wave lengths are able to precipitate the protein. The assumption that might be made in the case of diabetic cataract is that the sugar present in the humors of the eye modifies the lens protein so that the short wave lengths can bring about the precipitation. In other words, of two factors that may be involved in the production of cataract, ultra violet radiation and a modification of the protein, the latter factor is exaggerated in the production of diabetic cataract.

Glass blower's cataract, on the other hand, would seem to offer a case in which the radiant energy factor is increased. It is known that cataract occurs more frequently among glass blowers than among people generally. Crookes[5] found the radiation from molten glass in the glass blower's furnace to be very rich in red and infra red and for this reason he concludes that glass blower's cataract is due to the long wave lengths. He found that the radiation from the furnace was poor in short wave lengths. On the other hand Schanz and Stockhausen[6] found the radiation from molten glass in the glass blower's fur-

[4] These wave lengths were determined by Dr. F. M. Schultz of the University of Illinois.

[5] Crookes: Philosophical Transactions, Royal Society of London, 1914, A-509.

[6] Schanz und Stockhausen: v. Greafe Archiv f. Ophthal., 1910, lxxiii, 553.

nace to be especially rich in the region of the short wave lengths. Hence, contrary to Crookes' assumption, they conclude that glass blower's cataract is due to the short wave lengths. It seems to me that several objections could be raised to the assumptions of Schanz and Stockhausen as well as to those of Crookes. Experiments reported in this paper in which normal pig's lenses were exposed to infra red, red and ultra violet radiations without the production of opacity certainly do not bear out the conclusions of these investigators. The constitution of the normal human lens is more or less constant, the quality and quantity of radiation from the glass blower's furnace is more or less constant. If the radiation from the glass blower's furnace, whether it be infra red, red or ultra violet, be the only thing involved in the production of glass blower's cataract why is it that some glass blowers develop cataract while others do not? The fact that a large percentage of glass blowers do not develop cataract although their lenses are subjected to the same quantity and quality of radiation as in the case of those who do develop cataract would seem to imply the existence of another factor than the radiation. The fact that the normal lens protein cannot be precipitated by radiant energy while this normal protein can be so modified by chemical substances, similar to those found in cataractous lenses, that the short wave lengths in the spectrum become effective in this respect would appear to indicate as a second factor, a modification of the lens protein. The combination of the two factors named seems to offer an explanation of the fact that a relatively small percentage of glass blowers develop cataract while a large percentage although working under the same conditions do not develop it. It may be assumed that the relatively small percentage of glass blowers who develop cataract have a more or less disturbed condition of nutrition expressing itself in an increase of sugar, calcium, magnesium or some other substance which can so modify the lens protein that the short wave lengths of the spectrum are able to precipitate it. Assuming that nutritional disturbances are as frequent among workers in other occupations as among glass blowers the prevalence of cataract among glass blowers would

then be explained by the excess of the radiant energy factor. If these assumptions are true then a glass blower who has diabetes should develop cataract very rapidly. There are cases on record both among glass blowers and others where opacity of the lens once begun developed very rapidly. Unfortunately there are no data, so far as I have been able to find, which would connect these cases with the assumptions made so that the explanation suggested is based solely on the experiments reported in this paper.

The prevalence of cataract in the tropics has been noted frequently. For instance Colonel Henry Smith,[7] who kindly furnished the cataractous Indian lenses for the analyses referred to in this paper, has already performed in India more than thirty thousand operations for cataract. It may be recalled that the analyses of these lenses show the presence of a large amount of silicate. It will be remembered also that silicate is one of the substances which modifies the lens protein in such a way that the short wave lengths can precipitate it. It is known that tropical light is comparatively rich in ultra violet. A plausible explanation of the prevalence of cataract in India may be found in the combined effect of the presence of silicate in the lens and of the comparatively great amount of ultra violet radiation.

If the short wave lengths are permitted to fall on serum albumen or serum globulin, on egg albumen or egg globulin, vitellin, etc., they are absorbed and these substances sooner or later coagulate. The rapidity with which this coagulation takes place, other things being equal, depends upon the intensity of the ultra violet radiation. In view of the fact, that practically all proteins can be precipitated by means of the short wave lengths, the question naturally arises why is it almost impossible to precipitate the unmodified lens protein by similar wave lengths? The fact that it cannot be precipitated would point to an adaptive provision which needs explanation.

The lens possesses the property known as fluorescence, i.e.,

[7] Tiffany: Indian Medical Gazette, 1914, xlix, 326.

it absorbs the shorter waves and radiates this absorbed energy in the form of longer waves. It may be assumed that in this manner the lens disposes of more or less of the energy of the absorbed short waves and hence no coagulation of the protein occurs. I have found that those substances, calcium chloride, etc., which modify the lens protein in such a way that the short waves can precipitate it at the same time decrease the fluorescence of the lens. This observation lends support to the assumption that the fluorescing property of the lens protects its protein from precipitation by ultra violet radiation. However, I realize that this is merely a provisional hypothesis which must be tested by further experiments.

SUMMARY

1. Radiation from a quartz mercury vapor lamp which is sufficiently intense to coagulate egg albumen, egg globulin, vitellin, serum albumen and serum globulin in 1 hour does not coagulate the protein of the normal lens or of the vitreous or aqueous humors and hence does not affect the transparency of these structures after a continuous exposure of 100 hours.

The region of the ultra violet spectrum effective in coagulating the egg white lies between $265\mu\mu$ and $302\mu\mu$. The region most effective lies around $265\mu\mu$.

2. The lens protein can be modified by solutions of calcium chloride, magnesium chloride, sodium silicate or dextrose too weak of themselves to affect the transparency of the lens so that ultra violet radiation can precipitate the modified lens protein and hence produce opacity of the lens. The effective region in case of modification by calcium chloride is from $265\mu\mu$ to $302\mu\mu$ inclusive. The most effective region lies around $265\mu\mu$.

3. Analyses of senile cataractous human lenses show that calcium, magnesium, and in lenses from India, silicates are greatly increased in this type of cataract. The assumption is made that the accumulation of these substances modifies the lens protein in such a way that the short waves of the spectrum can precipitate the protein thus producing opacity or cataract.

4. The assumption is made in the case of diabetic cataract that the accumulation of sugar in the liquids of the body so modifies the lens protein that the short waves of the spectrum can produce opacity, hence the prevalence of cataract in this disease.

5. The above named substances which so alter the lens protein that the short waves can precipitate it at the same time decrease the fluorescence of the lens. This suggests that there may be some relation between this latter property and the great resistance of the normal lens protein to ultra violet radiation.

6. In looking for the cause of cataract it would seem that at least two factors are to be considered, the one, a modification of the lens protein, and the other, radiation of short wave lengths by which this modified protein can be coagulated.

7. Radiation from the infra red, or the visible regions of the spectrum cannot coagulate either the modified or the unmodified lens protein provided the coagulation due to heat be excluded.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XVII. On the Chemical Control of the Gastric Hunger Mechanism

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The gastric hunger contractions are inhibited by mechanical and chemical stimulation of the nerve endings in the mucous membrane of the mouth, the esophagus and the stomach.[1] This insures inhibition of the hunger contractions during mastication and gastric digestion. The gastric hunger mechanism receives motor or tonic innervation via the vagi. The central connections of this tonus innervation appear to be practically isolated from all normal reflexes, while the inhibitory mechanism via the splanchnic nerves is very rapidly called into activity reflexly.[2] The foregoing facts appear to have only two alternative explanations as regards the positive control of the gastric hunger mechanism, viz.,

1. The gastric hunger contractions are due to a specific automatism (central and peripheral) primarily independent of afferent impulses as well as the conditions of the blood. Such an automatism, would, of course, vary with the physiological condition of the automatic tissues; but if this is the mechanism we cannot speak of any physiological control of the hunger apparatus, except in the way of inhibition.

2. The central and peripheral tissues concerned in the genesis of the hunger contractions may be influenced in a positive way by physiological changes in the blood. If this is the case, we might

[1] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, pp. 245, 369, 389.

[2] Carlson: Ibid., 1914, xxxiv, p. 155.

expect such changes in the blood to be specially evident in the normal animal when starving.

Some facts already established seem to show that both of the above factors are to be reckoned with. In man and dog the gastric hunger contractions usually appear as soon as the stomach is empty of food, that is, before intestinal digestion and absorption of the meal is completed. Under these conditions the initiation of the hunger contractions must be due to a primary automatism, not opposed by inhibitory reflexes, rather than to any changes in the blood such as are presumably involved in starvation, for there is surely no auto-digestion of the body tissues or lack of pabulum in the body fluids, or while normal intestinal digestion and absorption is still in progress. In dogs with Pawlow stomach pouches we may also have hunger contractions in the main stomach while the Pawlow stomach is quiescent, or *vice versa*.[3] On the other hand, prolonged starvation,[4] and pancreatic diabetes,[5] which is a type of starvation, leads to increased activity of the hunger mechanism, at least up to the point where the stomach becomes directly involved in the general debility and cachexia. That increased vigor of the hunger apparatus is an after effect of a greatly accelerated metabolism is a bit of evidence pointing in the same direction.

This augmentation of the hunger contractions in starvation may be due to

1. The appearance of substances in the blood stimulating the central tonus mechanism or the peripheral hunger apparatus.
2. The absence or diminution of inhibitory substances in the blood.
3. The absence or depression of inhibitory reflexes.
4. Starvation changes in the tissues directly concerned in the hunger contraction.

If it is due to the presence of stimulating substances in the blood, it would seem that transfusion of the blood of starving

[3] Carlson, Orr and McGrath: Ibid, 1914, xxxiii, p. 119.

[3] Carlson: Ibid., 1914, xxxiii, p. 95; T. L. Patterson, experiments not yet published.

[5] Luckhardt: Ibid., 1914, xxxiii, p. 313.

animals into normal animals ought to augment the activity of the hunger mechanism, at least temporarily. We are now in position to report that this is actually the case.

THE TECHNIQUE OF THE TRANSFUSION EXPERIMENTS

Direct transfusion from the starved donor to the normal recipient by direct union of blood vessels is not feasible, because if this is done under general anesthesia, the anesthetic itself depresses the stomach, and if it is done with aid of local anesthesia only the recipient is so disturbed that the stomach is inhibited reflexly. But we found that good natured and gentle dogs used to our routine of recording the gastric hunger contractions were practically not disturbed at all by the puncture of the saphenous vein with a sharp needle and injecting 20–50 cc. fresh drawn and defibrinated blood. This technique was therefore adhered to. In the preliminary training of these dogs the animals' legs were handled in various ways (shaved, injection of salt solution, etc.), so that the animal finally paid little or no attention to the handling of the leg or the insertion of the needle into the vein. In some cases we decreased the sensitivity of the skin over the saphenous vein by the application of carbolated vaseline.

THE EFFECTS OF BLOOD FROM STARVED ANIMALS

The intravenous injection of 20–50 cc. of fresh defibrinated blood from starving dogs into normal dogs increases the gastric tonus and hunger contractions of the latter, if their stomachs are empty and moderate tonus and hunger contractions are in evidence in the recipient at the time of the injection of the blood. If the stomach of the recipient, although empty of food, is atonic and hunger contractions are completely absent at the time of the injections, the blood from starving animals has practically no action on the stomach. The stimulating action of this blood on the stomach already in moderate tonus and hunger contractions lasts from ten to thirty minutes, depending on the quantity of starved blood injected.

The above conclusion is based on 25 experiments on four gastric fistula dogs. The blood for the transfusion was drawn

from animals after five to twelve days of starvation. A typical tracing illustrating this stimulation of the hunger mechanism by small quantities of blood from starving animals is reproduced in figure 1A.

The failure of starved blood to induce tonus and hunger contractions in atonic and quiescent stomachs is probably due to the fact that by the present method of transfusion it is not possible to introduce enough starved blood to overcome the depressor or inhibitory factors responsible for the atonic and quiescent condition.

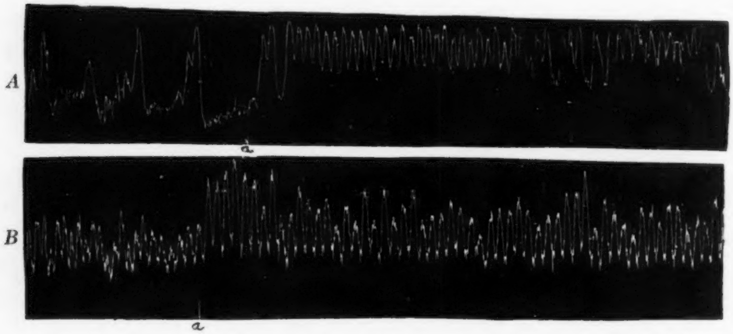


FIG. 1. TRACING FROM THE EMPTY STOMACH OF DOGS. (Reduced ⅔).

Chloroform manometer. *A*. At an intravenous injection of 35 cc. blood drawn from a dog on the eighth day of starvation. Showing stimulation of the gastric hunger apparatus, in the change from Type I to Type III hunger contractions (hunger tetanus). *B*. At an intravenous injection of 20 cc. of blood from a dog in pancreatic diabetes. Showing stimulation of the gastric hunger mechanism.

THE EFFECTS OF BLOOD FROM DIABETIC ANIMALS

Using the above technique 20–50 cc. of blood from animals in pancreatic diabetes and showing the typical diabetic polyphlagia were transfused into normal animals. The results were practically identical with those from the blood of starving animals, that is a temporary stimulation of the gastric hunger mechanism. A typical tracing showing this effect is reproduced in figure 1B.

CONTROL EXPERIMENTS

Twenty to fifty cc. of blood from normal dogs or from dogs during the height of digestion were transfused into dogs while their gastric tonus and hunger contractions were being registered. In the majority of these experiments the transfusion had no effect at all on the motor condition of the empty stomach. In a few cases it acted as a very slight and transient stimulus, but in no instance did the blood from normal animals produce the marked effects obtained from the blood of starving and of diabetic animals. Hence we conclude that the latter results are due to something in the blood of starving and of diabetic animals not present, or present in less concentration in the blood of normal animals. It is evidently not due to the transfusion of the above quantities of defibrinated blood as such. The intravenous injections of 20–50 cc. of 0.9 per cent NaCl is also without effect on the hunger mechanism.

It is well known that intravenous injections of considerable quantities of fresh defibrinated blood may cause temporary vasomotor and cardiac disturbances. Lowering of the arterial blood pressure is usually a feature of these disturbances. It is highly improbable that vaso-dilatation is a factor in the marked results produced by blood from starving and diabetic animals. The following control tests were made. One per cent peptone in 0.9 per cent NaCl was injected intravenously, and amyl nitrite was administered by inhalation. If sufficient peptone or amyl nitrite was given to affect the gastric tonus and hunger contractions, this effect was always in the direction of inhibition and paralysis. It is not clear, however, that this inhibition was due solely to the vaso-dilation, but the experiments show that a general vaso-dilation does not necessarily lead to stimulation of the gastric hunger apparatus.

THE ACTION OF THE ACETONE BODIES ON THE GASTRIC HUNGER MECHANISM

As a preliminary step in the analysis of the above stimulation of the gastric hunger mechanism by starved and diabetic blood, we have tested the action of acetone, and oxybutyric acid on the

gastric hunger contractions. We had also planned to use diacetic acid in these experiments, but we were not able to obtain this acid at that time. It is well known that prolonged starvation as well as diabetes leads to acidosis, although Mariott[6] has recently shown that there is practically no acidosis in pancreatic diabetes in dogs. It seemed possible that the acetone bodies might be the stimulating factors in the starvation and the diabetic blood. The action of the acetone bodies dissolved in Ringer's solution were tested on a number of animals with uniformly negative results. That is to say, the acetone bodies in concentrations that effect the gastric hunger apparatus at all, cause inhibition and depression. No indication of any primary or secondary stimulation by the acetone bodies could be secured. It is therefore clear that the stimulating action of starvation and diabetic blood on the hunger mechanism is not due, at least not directly, to the condition of acidosis of the blood.

THE EFFECT OF HEMORRHAGE ON THE GASTRIC HUNGER MECHANISM

It occurred to us that some of the conditions of starving might be produced temporarily by hemorrhage. It was recognized, of course, that hemorrhage also introduces factors not present, at least in moderate starvation, such as the temporary diminution of hemoglobin. Nevertheless, the results of two series of experiments with the effects of excessive hemorrhage were so striking and conclusive that they are reported here, even though we have not worked out their interpretation. The results are most conveniently stated by the following brief protocols:

October 20. Type II and III gastric hunger contractions.

October 21. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 22. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 23. Type I contractions. Gastric tonus equals 3 cm. chloroform.

[6] Marriott: Jour. of Biol. Chem., 1914, xviii, p. 507.

October 24. Type I contractions. Gastric tonus equals $2\frac{1}{2}$ cm. chloroform.

October 27. 9.12 a.m. light ether anesthesia, 146 cc. blood drawn from carotid artery at 9.30 a.m. Recording of the gastric hunger contractions began 10.08 a.m. At this time the stomach was atonic and quiescent. A gradual return of gastric tonus appeared at 10.30. At 11 a.m. the gastric tonus was 5 cm. chloroform with vigorous Type III hunger contractions, and this condition persisted till the end of the experiment at 12.30.

October 28. Type I contractions. Gastric tonus equals $2\frac{1}{2}$cm. chloroform.

October 29. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 30. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 31. Type I contractions. Gastric tonus equals $2\frac{1}{2}$ cm. chloroform.

Control Experiment on Dog I, November 18, Ether anesthesia for 20 minutes

November 18. Type I contractions (very feeble). Gastric tonus 2 cm. chloroform.

November 19. Type I contractions (feeble). Gastric tonus 2 cm. chloroform.

November 21. Type I contractions. Gastric tonus 2 cm. chloroform.

November 25. Type I contractions. Gastric tonus 2 cm. chloroform.

November 26. Type II contractions. Gastric tonus $3\frac{1}{2}$ cm. chloroform.

Dog VII. Weight 6.7 k.

October 30. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

October 31. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 3. Type I and II hunger contractions. Gastric tonus 3 cm. chloroform.

November 4. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 5. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 6. 9.10 a.m. 169 cc. blood withdrawn from carotid artery under light ether anesthesia. Record of gastric contractions began at 9.45. At this time the stomach was quiescent with feeble tonus. At 10 a.m. the gastric tonus began to increase. At 10.30 the gastric tonus was 9 cm. chloroform with Type III vigorous hunger contractions. This condition persisted till the end of the experiment at 11.30.

November 7. Type II and III contractions. Gastric tonus $2\frac{1}{2}$–3 cm. chloroform.

November 11. Type II and III contractions. Gastric tonus 3–7 cm. chloroform.

November 12. Type I contractions. Gastric tonus $2\frac{1}{2}$ cm. chloroform.

Control Experiment on Dog II. November 18, Ether anesthesia for 20 minutes

November 18. Type I and III contractions. Gastric tonus 1–4 cm. chloroform.

November 20. Type I and III contractions. Gastric tonus 2 cm. chloroform.

November 21. Type I contractions. Gastric tonus 2 cm. chloroform.

November 24. Type I contractions. Gastric tonus 2 cm. chloroform.

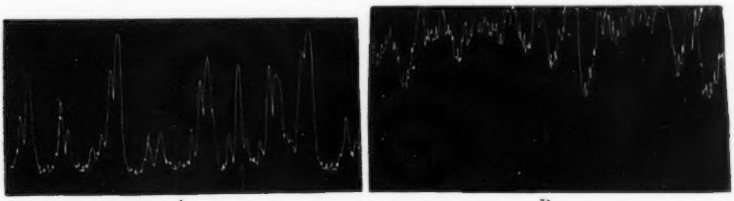
November 25. Type III contractions. Gastric tonus 3–4 cm. chloroform.

November 26. Type I and III contractions. Gastric tonus 3–6 cm. chloroform.

The reader will note that in both dogs the hemorrhage induced temporarily a greater gastric tonus and intensity of hunger contractions than typical for these dogs before the hemorrhage. This effect of the hemorrhage disappears in less than twenty-four hours. The controls show that the stimulation of the gastric tonus mechanism is due to the hemorrhage, and is not an after effect of the ether anesthesia. That they were felt as hunger contractions by the dogs was evidenced by the amount of food consumed on the hemorrhage days.

A typical tracing showing this stimulating action of hemorrhage on the hunger mechanism is reproduced in figure 2.

The following considerations might be offered not only as a possible but also as a probable explanation. The blood is, of course, the purveyor of nutritive substances to all the tissues of the body. Its chemical composition is kept remarkably constant. If now an animal is bled extensively (2–3 per cent of body weight) there is removed suddenly an enormous amount of pabulum, that is, of those *various* substances which are taken up by the *different* tissues during circulation. The organs and tissues deprived of these respective nutritive substances become hungry and give up a something (a hormone) to the circulation



A *B*

FIG. 2. (Reduced ⅗).

A. Tracing showing how gastric tonus and Type I hunger contractions characteristic of Dog VII before hemorrhage. *B*. Record of gastric tonus and hunger contractions of Dog VII sixty minutes after drawing 169 cc. blood from the carotid artery. Showing the temporary stimulation of the gastric hunger mechanism as an after effect of excessive hemorrhage. (Bottom of tracing = 0 mm. pressure).

which reaching the muro-muscular apparatus of the stomach stimulates the latter to the production of the hunger contractions.

We recognize, of course, that acute hemorrhage introduces other factors. Some of them have been mentioned. The explanation offered gives a simple and reasonable picture of the mechanism involved. By acute hemorrhage we induce suddenly temporary but *acute* starvation. Probably all the tissues of the body give up this "hunger hormone." By withholding food from the animal these "hunger hormones" accumulate more slowly, depending for one thing on the state of nutrition and reserve food supply of the animal before the period of actual tissue starvation begins.

SUMMARY

1. Blood from starving animals and animals in pancreatic diabetes transfused into normal animals acts as a temporary stimulus to the gastric hunger mechanism.

2. Excessive hemorrhage is followed by a temporary augmentation of the gastric hunger contractions.

3. Prolonged starvation, pancreatic diabetes, and *possibly* excessive hemorrhage result in the increase of some substance or substances in the blood that act as stimuli to the gastric hunger mechanism.

ON THE SECRETORY INNERVATION OF THE HYPOPHYSIS

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Cushing[1] and his co-workers have recently reported experiments that seem to indicate a secretory innervation of the hypophysis. Dandy[2] has reported histological studies of the nerve running up to the gland along the arterioles. He finds that these nerves are derived from the sympathetic carotid plexus. He is unable to make any absolute differentiation between secretory and vasomotor nerves, but he is inclined to the belief that these are secretory nerves, this inclination being based on the observation that these trunks make connections with other cranial nerves, that the anterior lobe has a richer nerve supply than the posterior lobe, and that no vasomotor nerves have yet been demonstrated in the cranial cavity.

Cushing attempted to discover the presence of secretory nerves to the glands by physiologic means. If a hyperplasia or diminished activity of the gland brings about adiposity and increased sugar tolerance, an excessive activity of the gland might bring about a decreased carbohydrate tolerance and give rise to glycosuria.[3] Cushing accordingly carried out a series of experiments on rabbits consisting of a prolonged electrical stimulation of the superior cervical ganglion with the animals under anesthesia. He found that a glycosuria invariably followed such a procedure, and he therefore concluded that "*the pituitary body, and more particularly its posterior lobe, plays a significant rôle in the metabo-*

[1] Weed, Cushing, and Jacobson: This Journal, 1813, xxxi, p. xiii.

[2] Dandy: Am. Jour. of Anat. 1913, xv.

[3] Borchardt: Zeitschr. f. Kl. Med. 1908, lxvi, p. 332; Goetsch, Cushing, and Jacobson: Bull. Johns Hopkins Hospital, 1911, xxii, p. 165.

lism of carbohydrates, and its action in this respect in under the control of fibers which reach the gland by way of the cervical sympathetic ganglion. Stimulation of this nervous pathway at this ganglion liberates a chemical substance which causes glycogenolysis and glycosuria independent of any possible nervous impulse reaching the glycogen-holding cells or the abdominal viscera."

Dr. Carlson suggested to us that the glycosuria obtained by Cushing may be due to other factors than the stimulation of secretory nerves to the hypophysis. In the first place prolonged anesthesia tends to induce glycosuria especially in rabbits and cats. Handling of the vagi is almost unavoidable during the operation and on stimulation of the superior cervical ganglion it is difficult to prevent escape of current to the vagus. Even partial exposing of the abdominal viscera while inserting cannulae into the ureters affects the normal state of the animal. The animal under stimulation is therefore far from being under a normal physiological state.

At the suggestion of Dr. Carlson we have repeated Cushing's work with various modifications. Our results seem conclusive; but it must be understood that the work deals with but one method of approaching the general problem, namely, that of the carbohydrate tolerance or rather glycosuria. It leaves open all other possible avenues of approach that might definitely prove or disprove a secretory innervation of the hypophysis.

EXPERIMENTAL PROCEDURE

Rabbits, cats and dogs were used in these experiments. Both cervical sympathetic trunks were isolated from the vagi, with as little handling of the latter as possible, and then cut, leaving a silk thread attached to the central end of each. This operation was, of course, carried out under light anesthesia and aseptically. The neck of the animal was wrapped carefully, but the wound was left open so that after the animal has been given ample time (8–24 hours) to recuperate and the post operative urine tested for sugar the two nerve trunks could be stimulated while the animal was conscious and normal. The dilatation of the pupils

was indicative of the activity of the nerves. This procedure permits stimulation of the possible nervous pathway to the gland similar essentially to that carried out by Cushing, while the animal is practically in a normal condition. After a continuous stimulation for a number of hours (1–3) the animal was put into a metabolism cage and the voided urine tested for sugar.

It was planned further that, if stimulation of the nerves under physiological conditions outlined above produced no glycosuria, two kinds of controls would be run. First, the crucial operation of Cushing would be exactly duplicated, but would not be followed by any stimulation of the superior cervical ganglia. The animal would be just allowed to lie with its tracheal cannula and the urine collected from time to time and tested for sugar. By the crucial operation is meant the isolation of the cervical sympathetic nerve from all vagus association and the subsequent isolation of the superior cervical sympathetic ganglion. The second kind of control would consist of merely inserting a tracheal cannula and then letting the animal lie undisturbed without any nerve handling or without their stimulation. Here, too, the urine would be tested from time to time for sugar. These two kinds of controls would determine in the first place the combined effects of operation and prolonged anesthesia, and in the second place the effects of mere anesthesia.

Next it was planned to ascertain if the gylcosuria obtained by Cushing was in any way facilitated by reflex vagus influence as a result of the handling or stimulation in the operation. For this purpose it was decided to section the splanchnics aseptically immediately below the diaphragm and, after the animal has recuperated sufficiently, to repeat the crucial operation., The cutting of the splanchnics would, of course, obviate later any possible reflex vagus influence on the adrenals or the liver.

Finally, if it were found that no glycosuria occurred as a result of stimulating the cervical sympathetic nerves under physiological conditions, it was planned to determine whether or not such stimulation was capable of producing a measurable hyperglycemia. For this purpose samples of blood would be drawn from the conscious animal at definite intervals before, during and

after stimulation of the nerves, and the sugar content then determined.

The administration of ether to rabbits and cats was carried out by means of a large bell jar so as to obviate excessive struggling and emotional glycosuria. Fifty to one hundred cc. of water were given by a stomach tube to insure the getting of urine. For the stimulation a tetanizing current was used. A tetanometer giving 3–8 stimuli per second was always placed in the circuit to prevent a too rapid bombardment of stimuli. During the stimulation the animal was kept in an oilcloth bag in such a way that merely the head and neck were free. This served to prevent struggling as well as a possible loss of urine. For the crucial operation a cannula was inserted into the urethra. An incision into the abdominal cavity very close to the symphysis oss. pubis was made just large enough to permit pulling out of the urinary bladder. This obviated any exposure or handling of the abdominal viscera which is quite unavoidable if cannulae are inserted into the ureters. The qualitative and quantitative determinations of the urine sugar were made by the Fehling's and Benedict's methods respectively. The blood sugar content was determined by the Rona-Michaelis method.

RESULTS

The following results were obtained from a series of experiments carried out on 15 cats, 4 rabbits, and 3 dogs.

Crucial Operation

This comprises a series of 6 experiments carried out on 4 cats, 1 rabbit and 1 dog. Cannulae were inserted into the trachea and urethra respectively. Each sympathetic nerve was traced up to the superior sympathetic cervical ganglion, which was separated from the vagus ganglion lying in the same sheath. Then the ganglia were stimulated alternately at intervals of one minute. One typical experiment is given in table I.

It is seen that here there was sugar in the urine at the end of 1½ hours anesthesia and operation, even before stimulation of

TABLE I

Cat 5

SAMPLES OF URINE COLLECTED	VOLUME	SUGAR	
	cc.	*per cent*	*gram*
Preoperative urine	105.0	0	0
From bladder at beginning of operation	3.2	0	0
During operation and before stimulation End of ½ hour anesthesia	2.5	8.3	0.210
End of 2½ hours anesthesia End of one hour stimulation	2.0	8.5	0.170
End of 3 hours anesthesia End of 1½ hours stimulation	1.5	6.0	0.090
End of 4 hours anesthesia No stimulation	1.0	4.3	0.043
End of 5 hours anesthesia No stimulation	1.5	2.7	0.030

the superior cervical ganglia and the percentage of sugar bears no relation to the stimulation. This is true of all the experiments in this group.

Physiological nerve stimulation with urine sugar tests

This group includes 10 experiments carried out on 3 rabbits and 6 cats. Both cervical sympathetic nerves were cut aseptically and stimulated after the animal had recovered from the operation and the post-operative urine had been tested for and found free from sugar. The animals were kept in the bag and held on the lap so that they lay comfortably and quietly while the nerves were being stimulated. The results are summarized in table II.

This table shows clearly that in all animals except Cat No. 1 there was never a trace of sugar in the urine resulting from stimulating both cervical sympathetic nerves while the animals were conscious and normal. Even in that exception (Cat No. 1) it cannot be stated with certainty whether the slight amount of sugar was due to the effects of the operation or to the stimulation, for no post-operative urine was obtained to be tested.

TABLE II

ANIMALS AND NUMBER	PREOPERATIVE URINE 24 HR. SAMPLE		POST OPERATIVE URINE 24 HR. SAMPLE		POST STIMULATION URINE 24 HR. SAMPLE		DURATION OF STIMULATION
	Volume urine	Sugar	Volume	Sugar	Volume	Sugar	
	cc.	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*hours*
Rabbit No. 1 (right sympathetic nerve)	10	0	11	0	11	0	2
Rabbit No. 1 (left sympathetic nerve)	70	0	50	0	25	0	$1\frac{3}{4}$
Rabbit No. 2 (both nerves)	100	0	70	0.47	80	0	$1\frac{1}{2}$
Rabbit No. 3 (both nerves)	40	0	20	0	70	0	$1\frac{1}{4}$
Cat No. 1 (both nerves)	85	0	none		60	0.96	1
Cat No. 2 (both nerves)	100	0	52	1.7	33	0	1
Cat No. 3 (both nerves)	none		33	0	50	0	1
Cat No. 4 (both nerves)	115	0	?	0	30	0	$1\frac{1}{4}$
Cat No. 5 (both nerves)	50	0	105	0	105	0	$1\frac{1}{2}$
Cat No. 6 (both nerves)	70	0	180	0	160	0	$1\frac{1}{4}$

Physiological nerve stimulation with determinations of the blood sugar[4]

Having failed to produce glycosuria by stimulating the cervical sympathetic nerves under physiological conditions, we next set out to determine whether this stimulation could at least produce a hyperglycemia which was either too fleeting or too slight for sugar to enter the urine. For this purpose we drew samples of blood from the tails of two cats before, during and after stimulation of the nerves. But we found that it was next to impossible to keep the cats from getting excited and struggling while the blood is being drawn. The excitement, of course, produces an emotional hyperglycemia, as shown by Cannon and his co-workers. To obviate this we inserted carotid cannulae into two cats so that blood could be drawn very quickly without disturbing the animal. We repeated this test on two dogs. The dogs did not struggle at all while the samples of blood were being drawn from the tail or from a leg vein. The results are given in table III.

[4] The blood sugar determinations were made by Mr. H. Ginsburg.

TABLE III

	DOG NO. 2	DOG NO. 3	CAT NO. 14	CAT NO. 15
Per cent of sugar in the blood before stimulation of both sympathetic nerves......	0.11	0.13	0.194	0.181
Per cent of sugar in the blood at the end of 1 hour's stimulation....................	0.10	0.10	0.143	0.180
Per cent of sugar in the blood at the end of 2 hours' stimulation....................	0.13	0.12		

It is quite clear from the foregoing table that there is *not even a perceptible hyperglycemia resulting from the stimulation of the cervical sympathetic nerves under physiological conditions.* The high sugar content observed in Cats Nos. 12 and 13 were undoubtedly emotional hyperglycemia, even though the animals did not seem frightened or restless.

Controls

This group comprises a series of experiments carried out on 5 cats and 16 dogs. They were intended to determine the effects of the operation, vagus handling, and prolonged anesthesia without any stimulation of the cervical sympathetic nerves or ganglia. In Cat No. 6 the crucial operation was exactly duplicated, except that the operation was not followed by stimulation. In the rest neither the nerves nor the ganglia were even exposed, but the animals were merely allowed to lie under anesthesia with tracheal and urethral cannulae, the urine being collected at definite intervals and tested for sugar.

The urine was collected from the excised bladders of 16 dogs that had been kept under ether anesthesia for two to four hours each. Upon an analysis the urine of 7 of these 16 dogs was found to contain sugar, the percentage ranging between 1.2 per cent and 1.6 per cent. In other words, 43.7 per cent of these dogs had a glycosuria resulting from prolonged ether anesthesia alone.

All of the cats showed glycosuria at the end of one to one and one-half hours ether anesthesia. In 4 of the 5 cats of this series the nerves or ganglia were not even exposed, and yet the sugar in the urine ran a similar curve. It began to rise gradually at

the end of one to one and one-half hours anesthesia, reached its maximum at the end of three to four hours, and then gradually declined. The fact that in the dog group this was by far not so pronounced points to the fact that this is largely a question of the degree of susceptibility of the species, the dog being more resistant than the cat and the rabbit.[5]

Possible vagus influence on the glycosuria in the crucial operations

The object of the experiments in this group was to determine whether the glycosuria following the stimulation of the superior cervical ganglia was due solely to anesthetic effects or to the combined effects of anesthesia and vagus exposure and handling while isolating the ganglia. For this purpose the splanchnics were cut aseptically in two cats immediately below the diaphragm so as to obviate later any possible reflex vagus influence on glycosuria production. Both animals were then given a few days to recover fully, and then the crucial operations were carried out in the ordinary way with the results shown in table IV.

It is evident from table IV that the vagus does not in any noticeable degree partake in the glycosuria production during the crucial operation. For here the glycogen-holding viscera were cut off from all reflex vagus influence, and yet the sugar ran the ordinary curve. Again this table shows clearly that sugar appeared in the urine before any stimulation of the cervical sympathetic system.

SUMMARY

1. Stimulation of the cervical sympathetic nerves while the animal is conscious and under physiological conditions does not produce hyperglycemia, glycosuria, or diuresis. This is true for dogs, cats and rabbits.

2. In all cases where the animal is subjected to a crucial operation and prolonged ether anesthesia, glycosuria appears whether or not the superior cervical ganglia are stimulated. A similar

[5] Carlson and Ryan: This Journal, 1908, xxi, p. 301.

TABLE IV

Cat No. 11

SAMPLES OF URINE COLLECTED	VOLUME	SUGAR		VARIATIONS IN MANIPULATION
	cc.	per cent	gram	
From bladder at beginning of operation	10	0	0	Preoperative
End of ½ hour anesthesia; during crucial operation	1½	0	0	During operation 1 hour.
End of 1 hour anesthesia; during crucial operation	1	2.8	0.028	
End of 1½ hours' anesthesia; no operation and no stimulation	1½	4.3	0.64	No operation or stimulation.
End of 2 hours anesthesia; End of ½ hour ganglia stimulation	2	4.75	0.095	
End of 2½ hours anesthesia; End of 1 hour stimulation	2	6.5	0.130	During 2½ hours of ganglia stimulation.
End of 3 hours anesthesia; End of 1½ hours stimulation	2	4.06	0.092	
End of 3½ hours anesthesia; End of 2 hours stimulation	1¾	4.7	0.082	
End of 4 hours anesthesia; End of 2½ hours stimulation	1	2.8	0.028	
End of 4½ hours anesthesia; No stimulation	1¼	2.8	0.035	No stimulation
End of 5 hours anesthesia; No stimulation	3	1.6	0.048	

glycosuria occurred when the animal was subjected to mere prolonged anesthesia without even exposing the nerves or ganglia.

3. Subjection to prolonged anesthesia after exclusion of all possible downward impulses to the abdominal viscera through vagus reflexes, with or without stimulation of the ganglia, led to a similar glycosuria.

4. The glycosuria of prolonged ether anesthesia runs a uniform course, the amount of sugar gradually rising to a maximum and then gradually declining. This course is not influenced by the stimulation of the cervical sympathetic nerves, or the superior cervical ganglia.

Our results go to show that the presence of secretory nerves governing the activity of the hypophysis cannot be demonstrated by the glycosuria or hyperglycemia methods. The glycosuria resulting from the stimulation of the superior cervical ganglia is undoubtedly due to the effects of prolonged anesthesia and not to an excessive activity of the hypophysis caused by stimulation of a secretory nervous pathway to the gland. As stated previously, this work concerns itself solely with but this single method, but it leaves open all other possible methods that might be attempted definitely to prove or disprove a secretory innervation of the hypophysis.

The nerve fibers to the hypophysis described by Dandy may be vasomotor nerve fibers. The fact that no vasomotor nerves have been conclusively demonstrated in the cranial cavity does not prove that such nerves are absent from a complex gland like the hypophysis. And the fact that the anterior lobe has a vastly richer nerve supply than the posterior lobe does not in the least prove that these nerves are of a secretory nature. In fact, Cushing favors the view that the posterior lobe is mainly concerned in the internal secretion playing a rôle in the metabolism of carbohydrates.

We wish to express our gratitude to Dr. Carlson for his valuable advice and kind supervision of this work.

STUDIES OF AUTONOMIC THRESHOLDS

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I. SMOOTH MUSCLE INNERVATED BY THE CERVICAL SYMPATHETIC

It is usually stated that similar tissues in different parts of the body vary with respect to the strength of stimulus required to arouse them to activity. Also it is generally admitted that in electrical stimulation of nerves, some require strong, others weak stimuli to excite activity in the tissues innervated by them. Hitherto the methods used for studying quantitatively the strength of stimulus have been unreliable. The advent of the Martin system of quantitative stimulation, permitted a greater degree of accuracy to be attained.

The present investigation was undertaken for the purpose of ascertaining whether the strength of stimulus necessary to provoke response of tissue supplied by autonomic fibers is essentially the same for all fibers, or whether it varied with respect to the tissue supplied, i.e., muscular or glandular. The Martin method[1] of quantitative stimulation has been used throughout the research. By this method it is possible to compare thresholds within the autonomic system itself as well as thresholds of the autonomic system with those of ordinary somatic nerves.

Inasmuch as no reliable method of quantitative study by means of faradic stimuli has been available before, it is not surprising to find a paucity of references to such studies in the literature. Occasionally a statement is seen that the secondary coil was in a certain position for one stimulation and in another position for a second stimulation. A brief consideration of the

[1] Martin: The Measurement of Induction Shocks, New York, 1912.

secondary positions noted, and of the fact that the primary current is practically neglected convinces one that the quantitative comparisons must be far from precise.

In this study the cervical sympathetic in the neck was used, and responses noted in three smooth muscle structures which it is known to innervate; pupil, nictitating membrane, and the blood-vessels of the nasal mucous membrane. Movements of the pupil and nictitating membrane were observed directly. Constriction of the nasal vessels was observed by a method modified from that described by Tschalussow.[2] This observer really made a plethysmograph of the nasal cavity. He packed the posterior nares with material soaked in vaseline, thus closing the nasal cavity posteriorly; one of the anterior nares was packed with the same material, and in the other was placed a hollow glass tube. This tube was connected by rubber tubing to recording apparatus. Tschalussow found the method eminently satisfactory for the problem which he was investigating. In the present research it became necessary in detecting thresholds to have a perfectly smooth line recorded in order to observe the slightest departure from it in the way of vasoconstriction. Tschalussow's technique was not so well adapted here because it gave an irregular base line instead of a smooth line. The irregularities in the line were due to respiratory movements of the soft palate and to swallowing movements.

Instead of applying a simple packing in the posterior nares, a brass rod about one-eighth of an inch in diameter was used (fig. 1). One end of the rod was bent almost completely over on itself and terminated in two rather sharp prongs directed backward. A wad of cotton was tied over these prongs and then soaked with vaseline. An adjustable crossbar was fastened on the straight end of the rod. After the animal was anesthetized the vaselined cotton on the prongs was inserted beyond the soft palate and pulled forward by traction on the rod until progress was stopped by plugging the posterior nares. Then the crossbar was adjusted to fit snugly behind the canine teeth. Next

[2] Tschalussow, M. A.: Archiv fur die gesammte Physiologie, cli, 1913, p. 524.

the mouth was closed and tied shut by heavy twine. This twine passed in front of the upper canine teeth, then back of the crossbar and around the animal's lower jaw. In this way the packing was securely fastened; and at the same time any variation in size of the nasal cavity through respiratory or swallowing movements acting on the soft palate was effectively shut off. Next one of the anterior nares was packed with vaselined cotton; then a glass tube was placed in the other and packed air-tight with vaselined cotton. The incisor foramina were closed with plasticine. The glass tube was then tied to the brass rod which extended about three inches beyond the animal's mouth. In this way the apparatus was firmly anchored. The glass tube was then connected by rubber tubing with a sensitive tambour. Constriction of the nasal vessels was indicated by a fall in the writing lever, vaso-dilatation was indicated by a rise in the

Fig. 1. Apparatus Used to Close the Posterior Nares

lever. The idea of Tschalussow to use the nasal cavity as a plethysmographic indicator is of great value. It is undoubtedly the most sensitive indicator of vaso-motor response that we possess.

Cats were used throughout this research. Some of them were anesthetized with urethane; others were anesthetized at first with ether and then rapidly decerebrated. One cervical sympathetic was cut and glass shielded electrodes[3] were placed on the nerve. These were connected with an induction coil calibrated according to Martin.[4]

Table 1 shows the results of determining thresholds and gives some idea of the range in different animals. The average thresh-

[3] Sherrington: Journal of Physiology, 1909, xxxviii, p. 382.
[4] Martin: Loc. cit.

old value for the pupils is seen to be 5.73 Z units of Martin; next in order is the nictitating membrane with an average of 6.34 Z units and then the nasal vasoconstrictors with an average of 7.89 Z units. It is readily seen that all three are of the same order of magnitude and the average of the three averages might be taken as approximately the threshold of the cervical sympathetic in the neck. It is 6.65 Z units. The corresponding β units of Martin were not estimated in all cases, but the average of those that were estimated fell in the same order. The averages were for the pupil 3.32 β units, for the nictitating membrane

TABLE I

PUPIL		NICTITATING MEMBRANE		NASAL VESSELS	
Z	β	Z	β	Z	β
1.68		0.97		2.10	
1.87		1.87		3.80	
2.00		2.00		4.00	
2.50		2.50		4.20	
2.75		2.83		5.00	
3.92		3.72		5.00	
4.00		4.00		7.40	
5.00		4.00		9.00	
8.40		10.30		9.60	
10.30		11.00		11.00	
11.00		15.40		15.40	
15.40		17.60		18.20	
Average....5.73	3.32	6.34	3.68	7.89	4.58

3.68 β units, and for the nasal vasoconstrictors 4.58 β units. The average ratio of β to Z in these experiments was 0.58. This is of particular interest because it is almost identical with the ratio (0.57) obtained by E. L. Porter[5] in a large number of experiments on peripheral nerves. In view of this evidence it is thought justifiable to calculate β units for the whole series on the basis of this ratio.

The threshold of the nasal vessels was estimated for the vasoconstrictors since these fibers have been shown[6] to be present in the cervical sympathetic.

[5] E. L. Porter: This Journal, 1912–13, xxxi, p. 149.

[6] M. A. Tschalussow: Loc. cit., p. 523.

Urethanized and decerebrate animals were strikingly similar in the responses obtained, but if ether was used it was found that a greatly increased strength of stimulus was necessary to reach the threshold. Thus observation 19 on March 24 in an urethanized animal gave a threshold of 4 Z units for the nasal vessels; on March 25, observation 25, the same threshold in an etherized animal was 26.5 Z units; and on April 3, in observation 21 (decerebrate cat) the threshold was 4.2 Z units. The thresholds for pupils and nictitating membrane were correspondingly high in etherized animals.

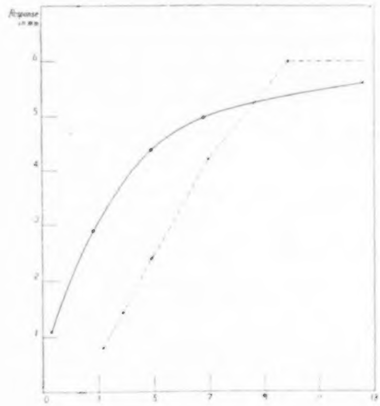

Fig. 2. Continuous curve shows response of nasal vessels plotted against stimulus strength (1.5 to 13 times the threshold). Dotted curve shows blood pressure changes in mm. of Hg plotted against strength of stimulus increasing from threshold (W. T. Porter).

Since a graphic record was kept of the response of the nasal vessels it was possible after the experiment to measure the degree of response and relate it to the strength of stimulus used to produce it. Figure 2 shows a curve in which strength of stimulus is plotted against degree of response. The abscissae represent the strength in terms of the threshold. Thus a stimulus whose strength is three times the threshold is called three. The or-

dinates represent the response in millimeters of change from the base line. No attempt was made to estimate the varying size of the nasal cavities so that the figures represent approximation only. Notwithstanding this fact there is a striking parallelism between this curve and the one plotted below with the interrupted line. This is a curve plotted by W. T. Porter[7] showing the response of the vasomotor center to increased strengths of stimuli. In the light of Dr. Porter's investigation, the nervous elements involved are conductors merely, and the bloodpressure change is an approximate index of contraction of bloodvessels. The two curves show a striking similarity of response of bloodvessels to increasing strengths of stimuli.

Another interesting fact brought out in this research is the close correspondence between the response of a peripheral autonomic fiber and the response in a reflex arc as found by E. L. Porter.[8] He found the average strength required for the flexion reflex to be 5.2 Z units. Combining the averages for pupil, nictitating membrane and nasal vessels in this investigation gives an average of 6.65 Z units for the cervical sympathetic. In the same investigation Porter showed an average of 2.3 Z units necessary to provoke response by stimulation of a peripheral nerve. The average β units for the cervical sympathetic was found to be 3.8. Porter found the average β units for the flexion reflex to be 2.7 and for peripheral stimulation 1.4. As previously stated he found the ratio of β to Z was 0.57; in this investigation it was 0.58.

It is very suggestive that the sympathetic in the neck should show a threshold equal practically to that of the flexion reflex. It is usually accepted as a fact that each synapse increases the resistance to the passage of an impulse, although the only quantitative evidence we have for that is shown by E. L. Porter's work. In his investigation as well as in this, at least one synapse was involved. Stimulation of the cervical sympathetic beyond the superior cervical ganglion seems to offer a means of determining whether the resistance resides in the synapse or in the

[7] W. T. Porter: This Journal, 1910–11, xvii, p. 278.
[8] E. L. Porter: Loc. cit.

peripheral organ. Some experiments which will be described in a subsequent paper are already planned for an answer to this question.

Another question suggested in this research is whether the three sets of fibers supplying nasal vessels, nictitating membrane and pupil really vary in their thresholds or whether the variation in thresholds is due to some peculiarity of the tissues in which the fibers end. This is a question which is pertinent to threshold values of any nerve and which must be determined by action current measurements. The point of practical importance to the physiologist is to know how much stimulus is necessary to apply to a nerve in order that it be physiological or at least comparable to normal stimulation.

SUMMARY

1. The application of quantitative stimulation to the autonomic system is described.

2. The threshold for contraction of an intrinsic smooth muscle of the eye (pupil) stimulated through the cervical sympathetic is 5.73 Z units, 3.32 β units.

3. The threshold for contraction of an extrinsic smooth muscle of the eye (retractor muscle of the nictitating membrane) stimulated through the cervical sympathetic is 6.34 Z units, 3.68 β units.

4. The threshold for contraction of smooth muscle of the nasal vessels (vasoconstriction) stimulated through the cervical sympathetic is 7.89 Z units, 4.58 β units.

5. The average threshold for the foregoing functions of the cervical sympathetic is 6.65 Z units, 3.86 β units.

6. The response to increasing strength of stimulus occurs in the order named, pupil, nictitating membrane, nasal vessels.

7. The degree of response of the nasal vessels is within limits directly proportional to the strength of stimulus.

The author wishes to express appreciation to Dr. E. G. Martin at whose suggestion this problem was undertaken and under whose supervision it was made possible.

THE CAROTID BLOODFLOW IN RELATION TO THE INTRA-ABDOMINAL PRESSURE

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Measurements of the bloodflow in the femoral vein have shown very clearly that the influx from the posterior extremities may be greatly altered by subjecting the intra-abdominal bloodvessels to different degrees of pressure.[1] Thus, it was readily possible to produce marked reductions in the femoral flow by moderately inflating the peritoneal cavity with air or by exerting gentle pressure upon the external surface of the abdomen. Quite similar results followed rather strong contractions of the diaphragm, induced by stimulation of the phrenic nerves.[2] No doubt, the descent of this membrane acts in the same way as the procedures just mentioned, all of them tending to lessen the vascularity of the abdominal viscera by first causing a greater venous discharge, which is followed later on by a reduction in the quantity of the blood normally entering the abdomen and posterior part of the body. As the venous channels are more readily compressible than the arterial, this reduction is ushered in by phenomena of venous hyperaemia and stagnation, while an arterial obstruction results only with high degrees of intra-abdominal pressure. Eventually these procedures lead to circulatory disturbances which are very similar to those resulting from occlusion of the abdominal aorta.[3]

[1] See Burton-Opitz, Pflüger's Archiv, cxxi, 1908, 156.

[2] See Burton-Opitz, American Journal of Physiology, vii, 1902, 435.

[3] Consult Sollmann and Pilcher, American Journal of Physiology, xxxi, 1912, 193.

In accordance with the results just mentioned it may justly be assumed that these impediments to the circulation through the posterior part of the body exert a profound influence upon the distribution of the blood as a whole by greatly augmenting the flow through the vessels of the head. To prove this contention, measurements of the bloodflow through the carotid and jugular systems of dogs were undertaken while different degrees of intra-abdominal pressure were established every now and then by inflation of the peritoneal cavity. The latter end was attained with the help of an ordinary trocar connected with an air pump, the degrees of pressure obtained being indicated by a manometer inserted in this circuit. The registration of the quantities of blood was accomplished by means of a stromuhr[4] inserted either in the left carotid artery or in the right external jugular vein. A membrane manometer connected with the central cannula of this instrument, served to indicate the bloodpressure.

Three experiments in all were made for the purpose of recording the changes occurring under these conditions in the carotid arteries, but as perfectly harmonious results were obtained, I may be permitted to shorten this discussion materially by considering in detail only a limited number of the phases of experiment 3, performed on February 27, 1914. The values of the bloodflow recorded during these periods, as well as other essential details, are contained in the accompanying table 1.

To begin with the bloodflow amounted on the average to 2.3 cc. in a second, while the pressure equalled about 108.5 mm. Hg Shortly after the beginning of phase 8 the abdominal cavity was inflated gradually, until the pressure therein rose to 20 mm. Hg, the inflation being continued during a period of about 70 seconds.

A comparison of the values of the bloodflow shows very clearly that the quantity of blood propelled during the inflation, is very much greater than that registered previous to this period of high intra-abdominal pressure. It is also evident that the augmentation begins almost immediately and gradually becomes more

[4] The recording stromuhr described by me has been used for this purpose. Pflüger's Archiv, cxxi, 1908, 150.

TABLE I

Carotid bloodflow in relation to intra-abdominal pressure. (Experiment 3, February 27, 1914)

Dog 12.5 Kg. Stromuhr in carotid artery

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE (CAROTID)	PROCEDURE
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	
1	8.8	22.8	2.59	105.0	None
2	10.7	22.5	2.10		
3	7.2	16.1	2.23		
4	8.0	19.2	2.41		
5	10.0	23.0	2.30	108.5	
6	8.3	20.3	2.44		
7	9.0	19.5	2.16		
Average			2.31	108.5	
8	7.7	20.5	2.66	125.0	Intra-abdominal pressure raised to 20 mm. Hg
9	7.3	20.0	2.74	138.5	
10	6.4	20.0	3.13	146.0	
11	4.7	17.2	3.66		
12	3.5	17.2	4.91		
13	3.6	17.5	4.86		
14	3.3	17.5	5.30		
15	3.7	20.5	5.54	140.0	
16	3.3	19.4	5.87		
17	3.0	17.4	5.60		
18	3.2	18.5	5.78	135.0	
19	3.2	18.2	5.68		
20	6.0	19.3	3.21	120.0	None
21	7.1	17.3	2.43	92.0	
22	8.0	17.5	2.18		
23	8.0	18.5	2.31	102.4	
Another procedure tested					
37	7.5	16.2	2.16	112.0	
38	8.0	18.5	2.31		
39	8.6	18.6	2.16		
40	5.6	18.9	3.37	121.0	Intra-abdominal pressure raised to 25 mm. Hg
41	4.8	18.0	3.75	130.5	
42	5.0	19.5	3.90		
43	4.5	19.4	4.31		
44	4.1	19.5	4.75		
45	4.2	20.0	4.76	125.0	
46	3.5	19.5	5.57		
47	3.5	20.2	5.79		
48	3.2	19.5	6.09		
49	3.4	19.2	5.63		
50	7.0	19.0	2.71	115.0	None
51	8.2	20.0	2.43	90.0	
52	10.8	19.6	1.81		
53	10.0	19.8	1.98		
54	10.5	19.2	1.82	95.5	

conspicuous in the course of the inflation, but naturally, a short time elapses before the maximal value of the flow is attained. The increase preserves in a measure a direct relationship to the degree of pressure exerted. In the experiments before us the flow is doubled with about 20 mm. of pressure and is rendered three times greater than normal by pressures ranging from 30 to 40 mm. Hg. Normal conditions are again established very shortly after the intra-abdominal pressure is permitted to return to its original low level.

This augmentation in the carotid flow is always accompanied by a rise in the general bloodpressure, the amplitude of which is in agreement with the amount of blood diverted to this particular region. It must be concluded therefore that this rise in the general pressure which is a familiar phenomenon to most of us,[5] finds its origin in the transfer of a large amount of blood from the circuits of the posterior part of the body into those of the head, forelegs and adjoining regions.

The foregoing phenomena may be rendered especially conspicuous by digital compression of the abdominal aorta below the diaphragm. It seems quite natural to suppose that this procedure possesses an influence upon the distribution of the blood which is very similar to that exerted by a high intra-abdominal pressure. While the posterior vascular channels are rendered more or less empty, the fore part of the body is made to accommodate an extra amount of blood.

A clear idea regarding the character of the changes occurring under these conditions can readily be obtained from the accompanying table II. It seems superfluous to insert a larger number of experiments, because the one here submitted fully proves the points previously emphasized. The carotid bloodflow amounted in this case to 2.24 cc. in a second, while the pressure continued at about 118.5 mm. Hg. The occlusion of the abdominal aorta raised the value of the bloodflow almost immediately to 4.68 cc. per second and the pressure to 145.6 mm. Hg,

[5] A very comprehensive study of intra-abdominal pressure has been made by H. Emerson. See: Archiv of Int. Med., vii, 1911.

these values being retained with slight fluctuations throughout the period of compression. On releasing the pressure upon the aorta normal vascular conditions were again established within a few seconds, but a very rapid decompression generally had the effect of causing a momentary subnormal flow and pressure.

To show that the variations in the carotid bloodflow are fully compensated for, the preceding tests have been amplified by

TABLE II

Carotid bloodflow on compression of abdominal aorta. (Experiment 1, May 5, 1914)

Dog: 14 Kg. Stromuhr in carotid artery

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE (CAROTID ART.)	PROCEDURE
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	
17	9.5	19.5	2.05	118.5	None
18	9.1	20.5	2.25		
19	8.2	20.6	2.51		
20	9.0	21.0	2.33		
21	10.1	21.0	2.07		
Average			2.24	118.5	
22	6.0	19.8	3.30	140.0	Compression of abdominal aorta
23	4.1	19.2	4.68		
24	4.8	19.4	4.04	145.6	
25	5.2	20.0	3.84		
26	9.8	20.4	2.08	102.8	None
27	10.0	18.8	1.88		
28	9.0	19.9	2.21	115.7	
29	9.1	18.9	2.06		
30	10.2	20.0	1.96		

a series of calibrations of the venous return through the right external jugular vein. In other particulars the experimental conditions are the same as those outlined previously. Experiment I, a part of which is presented in table III, is intended to portray the effects upon the venous influx of increasing the intra-abdominal pressure and experiment II, outlined in table IV, the changes following the digital compression of the abdominal aorta. In examining these data it should be borne in mind that the arterial pressure has been recorded in these experi-

TABLE III

Venous influx in relation to intra-abdominal pressure. (Experiment 1, May 8, 1914)

Dog: 12 Kg. Stromuhr in ext. jug. vein

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE		PROCEDURE
				Ext. jug. v.	Fem. art.	
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	*mm.* Hg	
12	12.0	18.9	1.57	0.8	108.4	
13	12.4	19.6	1.58			
14	11.2	19.6	1.75			None
15	9.9	19.7	1.99			
16	12.0	19.4	1.61		107.2	
Average			1.70	0.8	107.2	
17	8.8	19.8	2.25		118.1	
18	9.0	20.0	2.22		124.5	Intra-abdominal pressure raised to 30 mm. Hg
19	5.7	19.9	3.50	4.5		
20	8.0	19.9	2.48			
21	4.0	18.8	4.70	9.8	114.6	
22	8.0	18.9	2.36			
23	6.9	19.3	2.79	0.8	107.2	
24	8.9	19.2	2.15			
25	7.8	19.2	2.46			
26	10.2	19.5	1.91			None
27	10.4	18.5	1.77			
28	12.0	18.4	1.50	0.8	109.5	
29	14.0	19.0	1.35			
30	13.2	19.2	1.45			
31	12.4	19.2	1.54			
32	9.6	18.9	1.96	3.0	122.5	
33	7.4	19.4	2.62		130.0	
34	7.5	20.2	2.69			Intra-abdominal pressure raised to 30 mm. Hg
35	6.0	20.0	3.33	6.0		
36	5.4	19.5	3.61			
37	6.0	19.6	3.26	8.0	120.0	
38	7.5	18.6	2.48	1.0	109.0	
39	8.4	18.8	2.23			None
40	10.4	19.0	1.82			
41	12.6	19.0	1.51			

ments in the femoral artery and the venous pressure in the right external jugular vein. In order that the reader may be enabled to orient himself more fully, a limited number of the phases of each experiment have been reproduced in the text, the values given in the tables being directly transferable to the curves. In both reproductions the arterial pressure (*F*), as well as the

TABLE IV

Venous influx on compression of abdominal aorta. (Experiment 2, May 15, 1914)

Dog: 13.0 Kg. Stromuhr in ext. jug. vein

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE		PROCEDURE
				Ext. jug. v.	Fem. art.	
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	*mm.* Hg	
17	11.0	18.6	1.69	1.0	79.5	None
18	10.2	18.8	1.84			
19	11.4	18.9	1.65			
20	11.0	20.0	1.81			
21	9.6	20.1	2.09		77.2	
Average			1.86	1.0	77.2	
22	7.6	19.4	2.55	2.0	0.0	Compression of abdominal aorta
23	4.0	19.2	4.80			
24	6.0	19.8	3.30			
25	8.1	19.8	2.44	0.2	69.4	None
26	11.9	20.0	1.68			
27	11.0	19.8	1.80	0.8	78.1	
28	12.0	19.4	1.61			
29	11.4	19.6	1.71			
30	8.0	19.2	2.40	3.5	0.0	Compression of abdominal aorta
31	4.6	18.8	4.08			
32	4.0	18.2	4.55	4.0		
33	5.3	19.8	3.73			
34	8.1	19.0	2.34	0.5	65.0	None
35	12.6	19.8	1.57	1.0	82.0	
36	12.0	19.4	1.61			

venous pressure (*V*), has been recorded above the common abscissa *E*. The time (*T*) is given in seconds. In figure 1 the letters *AB* indicate the period of high intra-abdominal pressure and in figure 2 the time of compression of the abdominal aorta. The record of the stromuhr is marked by the letter *S*.

Fig. 1. Intra-abdominal pressure in relation to venous influx. (Curve reduced to 50% its original size.)

These later experiments prove very clearly that the venous bloodstream is subject to the same changes as the arterial, because the inflation of the abdominal cavity, as well as the compression of the aorta, produced increases in the venous flow in no way less pronounced than those encountered on the arterial side. A brief reference to the adjoining curves will prove this point. On examining figure 1, it is found that the bloodflow which previous to the inflation of the abdominal cavity amounted to only 1.70 cc. in a second, increases steadily as the pressure rises, until it reaches its maximal value of 4.70 cc. in a second during phase 21. It is to be noticed that this point coincides with the greatest degree of intra-abdominal pressure attained in this case. The venous pressure shows a rise which is in harmony with the increase in the bloodflow. Its value at the beginning of the experiment was 0.8 mm. Hg and at the end of the inflation 9.8 mm. Hg.

The general pressure, determined in the femoral artery, also presents a rise; eventually, however, its general level declines somewhat below that attained at the beginning of the inflation. This premature decrease implies that the high intra-abdominal pressure serves as an impediment to the flow of the blood to the posterior extremities, but naturally, the degree of pressure here employed (30 mm. Hg) is not sufficient to cause a blocking of the arterial inflow which would permit the femoral pressure to fall below its normal level. In this connection, it must be remembered that the pressure in the carotid system pursues a radically different course. Not being directly exposed to the high intra-abdominal pressure, the carotid arteries remain overfilled throughout the period of inflation and hence, the pressure within these bloodvessels retains its high level rather persistently, an appreciable compensatory fall occurring only if the experiment is continued for a relatively long time.

The phenomena displayed by figure 2 are very similar to those just described. The compression of the abdominal aorta occurring between points *A* and *B*, increased the venous flow from 1.86 cc. in a second to 4.80 cc. in a second. A marked rise in the venous pressure accompanied this change, while the general pressure which was determined in this case in the fem-

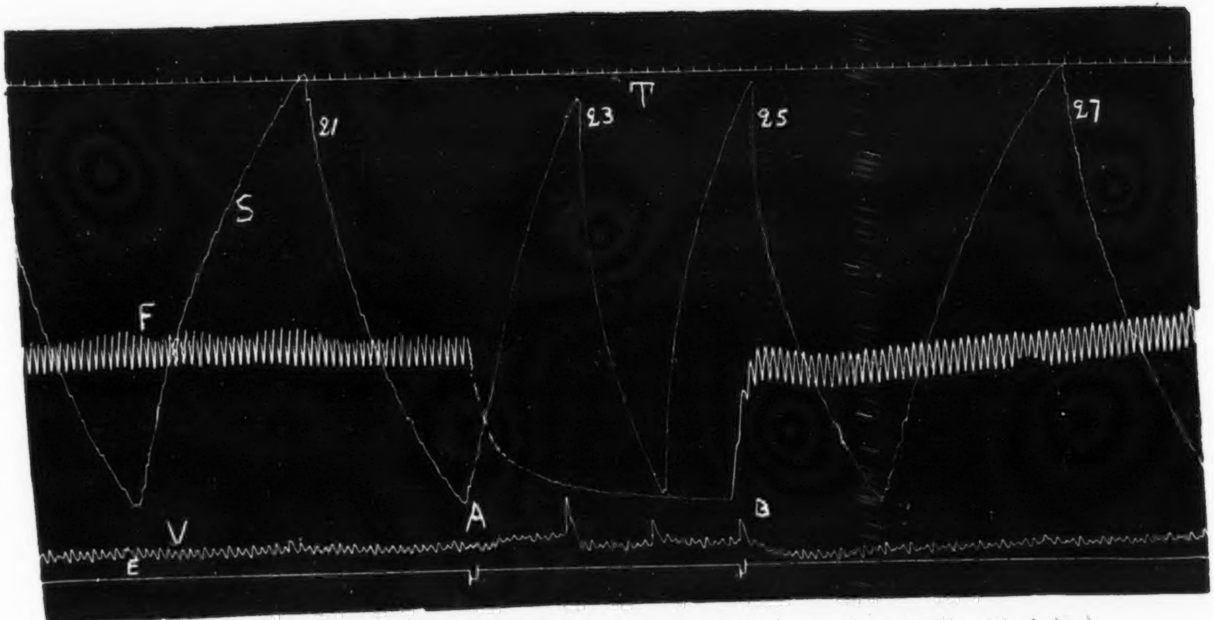

Fig. 2. Compression of abdominal aorta on venous influx. (Curve reduced to 80% its original size.)

oral artery, presented at this time a pronounced fall for obvious reasons. This curve also shows a compensatory fall in the pressures and the flow subsequent to point *B*, which was caused, as has been stated previously, by releasing the compression too suddenly.

The experiments briefly referred to at the beginning of this paper have shown that the downward movement of the diaphragm occasioned by the stimulation of the phrenic nerves, possesses a rather perplexing influence upon the distribution of the blood, in that it lessens the venous return from the posterior extremities and increases the flow from the external jugular veins. Clearly, this phenomenon cannot be explained satisfactorily upon the basis of an inspiratory fall in the intra-thoracic pressure, because the aspiratory force does not act in the same way upon the two venae cavae. A much more plausible reason for it is to be found in the increased intra-abdominal tension coincident with the descent of the diaphragm; which hinders the flow of the blood through the posterior channels of the body and greatly favors the circulation through bloodvessels of the head.

The question of whether an appreciable transfer of blood also takes place when the respiratory motions are shallow and of brief duration, cannot be answered with certainty. The present experiments suggest, however, that this mechanism is brought into play as soon as the movements become deep and prolonged or whenever an undue resistance is encountered by the descending diaphragm.

While clearly recognizing the important bearing of the intra-abdominal pressure upon the distribution of the blood, our attention must also be directed to the influence of the suction action of the inspiratory motions upon the venous return and especially upon that from the fore part of the body. Henderson and Barringer[6] seem to attach only a slight importance to this factor, because they state that "the suction induced in the intra-thoracic veins by the negative pressure of the thorax has by some writers been supposed to draw the blood onward from the extra-thoracic vessels. If so, the inspiratory increase of the

[6] Henderson and Barringer: American Journal of Physiology, xxxi, 1912, 402.

negative pressure would augment the venous supply to the right heart. It seems now to be generally recognized, however, that a suction cannot be transmitted to any considerable distance through the veins. They are too readily collapsible; the pressure within them is too low and the flow is too slow." It seems to me that these conclusions are based solely upon evidence which, at best, could be true only in a relative way and hence, it must be regarded as doubtful whether the factors here cited are sufficiently powerful to prevent an influence of this kind from being brought to bear upon the venous current.

I may be permitted to refer in this connection to certain experiments of my own[7] which, although not mentioned by the authors just cited, possess a direct bearing upon this question. These tests have shown that the respiratory motions induce inspiratory augmentations and expiratory retardations in the venous flow in the external jugular vein which preserve a direct relationship to the depth of the movements, and, that similar variations accompany the contractions of the diaphragm when induced by moderate stimulations of the phrenic nerves. Thus, having proved that the influences dependent upon changes in intra-thoracic pressure, must indeed be reckoned with, the suppositions of Henderson and Barringer cannot be considered as valid.

As these respiratory variations occur at intervals without that the venous flow suffers a marked retardation and, moreover, as the flow continues unabated when the respiratory movements are made to cease during brief periods of time, I felt justified in concluding further that the heart is by far the most important factor while respiration plays only a secondary part. The latter merely assists in propelling the blood into the more central venous channels. Henderson and Barringer[8] state that "according to our view the utmost assistance that respiration can afford to the circulation is to maintain a venous pressure sufficient to distend the right ventricle as rapidly as it closes and as fully as the duration of diastole allows." I confess that, in consideration of the experimental data submitted by me

[7] Burton-Opitz: Am. Journ. of Physiol., vii, 1902, 435.

[8] Loc cit., p. 400.

twelve years ago, I cannot do otherwise than fully coincide with this statement.[9]

The importance of the respiratory movements increases with every increase in the frequency and depth of the motions and hence, a state of activity may be reached at times during which the respiratory effects become indeed of surpassing importance to the venous flow and pressure. The evidence supplied by the present experiments therefore seems to favor the view that the chest and abdomen may act as a force and suction pump.

The question of whether a greater quantity of blood is furnished to the heart during inspiration, cannot be answered with certainty, because it must be considered as possible that the augmented flow from the fore part of the body is counterbalanced by a decrease in the influx from the inferior vena cava. Eppinger and Hofbauer[10] have pointed out that the lumen of this bloodvessel is lessened during inspiration. Under ordinary conditions this change would serve as an impediment to the venous return. While proving this supposition regarding the flow to be correct, the preceding tests have also shown that the period of lessened influx during the descent of the diaphragm is ushered in by a brief augmentation which may partially compensate for the decrease occurring later on.

For this reason I am rather inclined to believe that, on the whole, the cardiac vestibule and central venous channels receive a greater supply of blood during the inspiratory movement. Providing that the heart is sufficiently receptive at this time, it may be conjectured that the degree of venous pressure existing at the end of inspiration greatly favors an augmented influx into the auricle. But again, it is conceivable that the inspiratory and expiratory variations in the flow are counterbalanced and that the flow through the auricular orifice is therefore constant. I mention this possibility merely to show that a constancy of flow, such as Henderson and Barringer, as well as Piper,[11] assume to be present, could also be obtained without taking the "effective pressure" into consideration.

[9] See Burton-Opitz, This Journal, vii, 1902, 446.
[10] Eppinger and Hofbauer: Zeitschr. f. klin. Med., lxxii, 1911, 154.
[11] Piper: Archiv f. Anat. u. Physiol., 1913, 396.